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Original Articles

STUDIES ON BLOOD HISTAMINE IN CASES OF ALLERGY

I. BLOOD HISTAMINE DURING WHEAL FORMATION

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THE problem of the mechanism of allergic phenomena has interested many observers (Lewis, 1927, and Parrot, 1938). Much of the work has been concerned with the production of wheals, and, as with other allergic manifestations, histamine has been considered as a possible basic factor. Although early observations showed that a similarity existed between the wheal produced by an injection of histamine and that occurring in the skin of patients with urticaria or dermatographia (Eustis, 1912; Eppinger, 1913), the first thorough investigations were carried out by Lewis (1927) and his co-workers. It was shown that the wheal produced by scratching the skin of a sensitive person (dermatographia) was identical in size and in time relationships with that produced by the introduction of a small amount of histamine. Lewis postulated that, as a result of cell irritation caused by stroking the skin of a patient with dermatographia, the interaction of the antigen and antibody in a patient with urticaria, or physical irritants such as heat or cold in sensitive persons, a histamine-like substance was released at that particular site and was responsible for the production of the wheal.

Many observations tending to substantiate the histamine theory of wheal formation have been made. It was noted that the production of wheals in a patient with dermatographia was attended by a marked secretion of free hydrochloric acid into the stomach (Kalk, 1929). In 1936 Horton, Brown, and Roth reported on twenty-two patients sensitive to cold in whom symptoms ensued similar to those of histamine intoxication. These workers later showed that a release of free hydrochloric acid into the stomach occurs when one extremity of a cold-

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sensitive person is immersed in cold water, and that this release, along with the systemic reaction which follows, is identical with that occurring after the subcutaneous injection of histamine (Roth and Horton, 1940).

Proof of a more direct nature was furnished by the work of Rosenthal and Minard (1939), who demonstrated that histamine could be released from isolated pieces of human skin by electrical stimulation. Thus it would seem that histamine or some allied substance may be one of the factors responsible for the production of the symptoms.

In order to investigate this problem further, it was decided to study the blood histamine in a group of normal persons and in a series of patients with allergic manifestations. A total of 116 patients representing various types of allergy was studied. The present paper deals with a group of these in whom wheals could be produced readily by exposure to a specific stimulus. It was thought that, if histamine were released following skin stimulation, an increase of the blood histamine might be detected. Whether "physical allergy" differs basically from other types of allergy is still an open question, although the consensus of opinion is that the former is not founded on an immunologic mechanism. The present investigation deals only with the end phenomenon or wheal formation.

METHODS

The blood histamine was determined by the Code (1937) modification of the method of Barsoum and Gaddum (1935). Since it has been shown that prolonged stasis will increase the blood histamine (Barsoum and Smirk, 1936), blood was withdrawn from the antecubital vein only after removal of the tourniquet. The blood was then extracted and assayed on the guinea pig ileum suspended in Tyrode solution at 38° C., to which atropine was added in a concentration of 1:10,000,000. When this method is used, within 95 to 98 per cent of the histamine added to the blood is recovered.

In previous publications the histamine values have been expressed as histamine base in gamma per cubic centimeter; y = gamma or 0.001 mg. It would seem more logical if this were expressed as gamma per 100 c.c., and it has been decided to adopt this mode of expression in this and subsequent publications.

RESULTS

In previous observations on a group of fifty normal persons (Rose and Browne, 1940), it was shown that 4.0 y/100 c.c. (0.04 y/c.c.) is the average histamine content of whole blood, with variations of from 2.0 to 75.0 y/100 c.c. (0.02 to 0.75 y/c.c.). It was further noted that on repeated determinations the blood histamine tends, with minor variations, to remain remarkably constant in the same person. These results agree in general with those of Haworth and MacDonald (1937).

Effect of Stimulation of the Skin in Sensitive Patients.—A group of fifteen patients with various types of skin sensitivity were available for study. Of these, there were ten cases of dermatographia, three of cold allergy, and one each of heat and photosensitivity. Most of these patients responded with wheal formation when they were exposed to the specific stimulus. As a general rule, the blood histamine was determined once or twice beforehand on different days. The following procedure was then carried out. A control blood sample was taken, and then, depending upon the type of individual sensitivity, an area of the body was subjected to scratching, heat, cold, or ultraviolet light. For example, in the patients with dermatographia an area extending from the nape of the neck to the waist was scratched for five minutes. A forearm was immersed in water for five minutes at 5° C. in the cold-sensitive patients, and at 48° C. in the heat-sensitive group. The back of the photosensitive patient was exposed to ultraviolet light for three minutes. In each case blood specimens were again taken five, fifteen, and thirty minutes, and occasionally one hour after the stimulus was initiated. The blood samples were taken from either arm except in the cases where an arm was immersed in water; then the blood was taken from the stimulated arm. The results are given in Table I.

Of the ten cases of dermatographia studied, it will be observed that there was a well-marked increase of the blood histamine in Cases 49 and 51 only. A slight increase occurred in three cases (97, 112, and 218), and in the remaining five cases no change in the blood histamine was observed although whealing was readily produced. It will be noted furthermore that this increase of blood histamine appeared within the first fifteen minutes after the application of the stimulus and that the blood histamine had usually fallen to a normal level by the thirty-minute period. Case 63 was a normal person. There was very little skin response to the scratching, and there was no change in the blood histamine.

Somewhat similar findings were observed in the small group of patients with physical allergy, the results of which are given in Table I also. A moderate but definite increase in the blood histamine occurred in Cases 48 and 238, both patients with cold allergy. Case 58 is interesting in that an increase of the blood histamine occurred when the patient was exposed to either heat or cold. The reaction to hot water, however, was much more marked, for when she was immersed into a hot bath, there developed syncope, a marked lowering of the blood pressure, and involuntary micturition and defecation. The symptoms came on in about twenty to twenty-five minutes, and it will be observed that the blood histamine had fallen to below the original level. No change in the blood histamine was noted in Case 217, a patient with cold allergy, or in Case 212, one with urticaria solaris. In the latter, a marked response consisting of redness and swelling was produced by

TABLE I

BLOOD HISTAMINE IN CASES OF DERMATOGRAPHIA AND PHYSICAL ALLERGY FOLLOWING STIMULATION OF THE SKIN

NO. CASE	AGE (YR.)	SEX	DIAGNOSIS	BLOOD HISTAMINE IN γ/100 C.C.					DATA
				CONTROL	5 MIN.	15 MIN.	30 MIN.	1 HR.	
15	24	F	Dermatographia	1.5	2.5	1.2			Marked response, with whealing, flushing of the face, increased pulse
49	25	F	Dermatographia	7.0	40.0	10.0			Marked response, with whealing, etc.
51	48	F	Dermatographia	8.0	16.0	2.0	5.0		Marked response, with whealing
61	20	F	Dermatographia	3.5		3.5	3.5		Mild response
74	53	M	Dermatographia	4.0	4.0	4.0			Mild response, with wheal formation
97	19	F	Dermatographia	7.0	8.0	10.0	4.5	2.0	Moderate response, with wheal formation
112	9	M	Dermatographia	4.0	5.5	4.0	4.0		Mild response, whealing
121	32	F	Dermatographia	6.0	6.0	6.0			Marked response, wheal formation, and flushing of face
151	22	F	Dermatographia	6.0	6.5	5.0	6.0		Marked reaction and wheal formation
218	25	F	Dermatographia	4.0	5.5	4.0	4.0		Marked reaction, flushing of face, and whealing
63	24	M	Control	7.0	7.0	7.0	7.0		Erythema and flare; no whealing
48	24	M	Cold allergy	3.0		14.0			Arm placed in ice water at 5° C. for five minutes; swelling marked
58	57	F	Heat allergy	6.0	9.0	5.0	3.8	3.5	Patient immersed in hot water (48° C.) for ten minutes. Marked reaction with swelling
58	57	F	Heat allergy	10.0	14.0	10.0	10.0		Arm immersed in water at 5° C. for five minutes. Mild reaction with redness but no swelling
212	25	F	Photosensitivity	5.0	3.8	6.5	5.0	4.5	Exposed to ultraviolet irradiation for three minutes. Marked reaction with swelling
238	20	F	Cold allergy	4.5	4.8	6.0	3.8		Arm immersed in water at 5° C. for five minutes. Moderate reaction, no swelling
240	37	F	Control	7.5	7.0	8.0	8.5		Arm in water at 5° C. for five minutes. No swelling, mild erythema

exposure of an area of the back to an ultraviolet lamp for three minutes. Patients 88 and 240 were normal persons.

A secondary decrease in the blood histamine to below the original level was observed in a small number of patients of both groups (Cases 51, 97, 238, and 58). This is illustrated in Fig. 1. This decrease did

not occur as a general rule until twenty to thirty minutes after the stimulus had been administered and wheal formation had been present for some time. It was not observed in any case of physical allergy or dermatographia unless there first occurred an initial rise in the blood histamine. Case 58 is interesting in this respect. It was previously

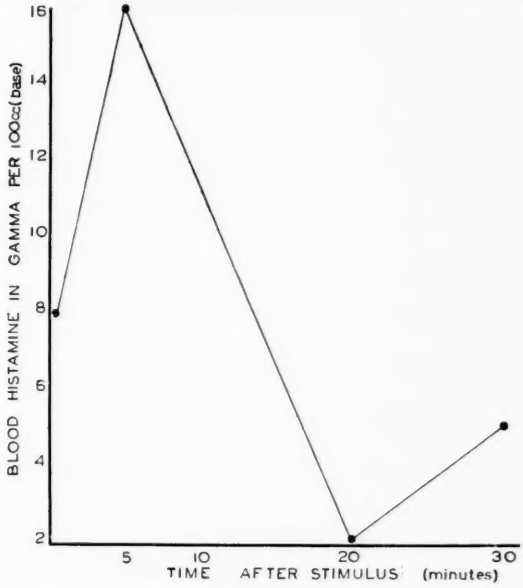

Fig. 1.—Changes in the histamine content of the blood in Case 51, female, aged 45 years, with marked dermatographia following stimulation of the skin of the upper back by scratching, which was followed by wheal production.

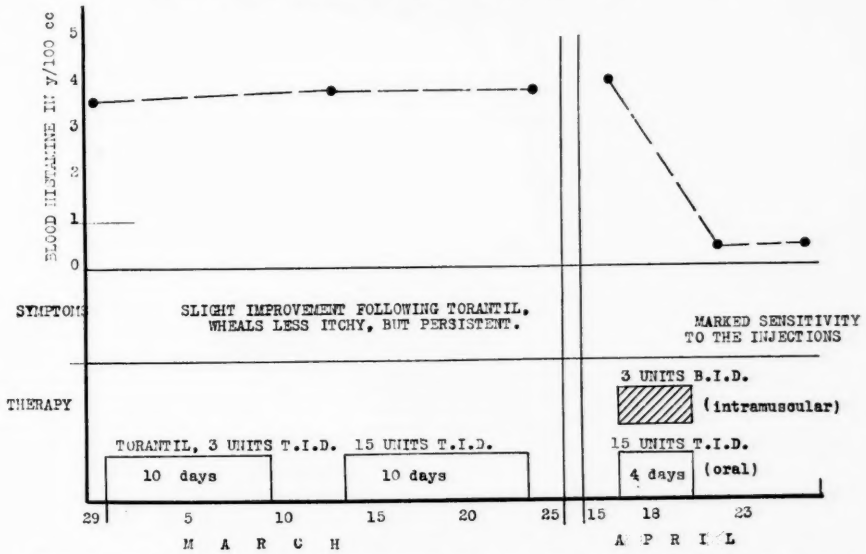

Fig. 2.—Blood histamine determinations in Case 133, female, aged 38 years, with chronic urticaria. It will be noted that the histamine content of the blood remained fairly constant until a marked reaction to the torantil was produced. The blood histamine was determined on two occasions when the arm in which the injection had been given was markedly swollen. The blood histamine was found to be at a low level.

noted that a release of histamine occurred when this patient was exposed to either heat or cold. Symptoms such as flushing, syncope, and the formation of edema occurred only when she was exposed to heat, and it will be noted that there is a decrease in the blood histamine at the time of the height of the symptoms.

A decrease in the blood histamine was noted in two additional patients. A patient (Case 133) who developed a sensitivity to intramuscularly injected torantil* was under observation for urticarial attacks. Several blood histamine determinations were made, and the blood histamine level was found to be 35 y/100 c.c. One-tenth cubic centimeter of a solution of torantil was injected subcutaneously, and within twenty-four hours the left arm became markedly swollen. The blood histamine was again determined and found to be at a low level (0.5 y/100 c.c.). (Fig. 2.) A similar observation was noted in Case 99, a young male found to be sensitive to sulfamethylthiazole. A blood histamine level was taken and found to be 8.0 y/100 c.c. The drug was given by mouth, and a second blood histamine level determined within fifteen minutes. No change was noted. In one hour, however, wheals had begun to appear on the skin, and the blood histamine level was found to be 4.5 y/100 c.c. (Fig. 3.)

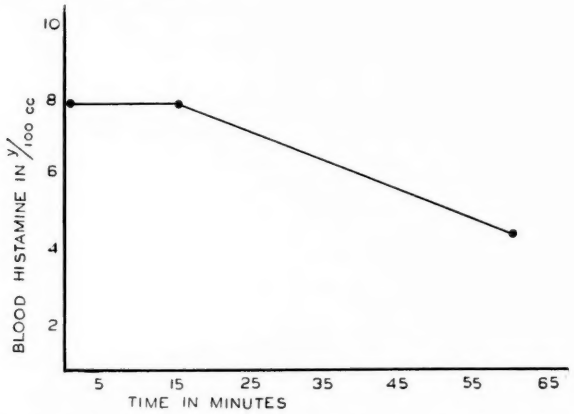

Fig. 3.—Change in the histamine content of blood in Case 99, male, aged 10 years, known to be sensitive to sulfamethylthiazole. A control blood sample was taken, and the drug was administered by mouth. Fifteen minutes after administration a second blood sample was taken, and no change was noted. Within one hour, however, urticaria had begun to form, and the histamine content of the blood taken at this time was found to be decreased.

DISCUSSION

From the results of the present experiments it appears that the histamine content in the peripheral blood may rise after skin stimulation in certain sensitive persons. This is presumably due to a release of histamine from the stimulated area. Slight variations were noted in the patient with hypersensitivity to ultraviolet light. Recently, how-

*Torantil, a histaminase preparation, was generously supplied through the courtesy of the Winthrop Chemical Co., and Dr. H. Cave, Montreal.

ever, an increase in the blood histamine of a light-hypersensitive patient following exposure has been reported by Capps and Young (1940).

Since histamine disappears rapidly from the blood (Rose and Browne, 1938) and since the increase observed in these experiments occurred within fifteen minutes following administration of the stimulus, it is probable that the release of histamine is rapid and that it remains in the circulating blood for a very short time. On the other hand, failure to detect an increase in the histamine content of the circulating blood following stimulation of the skin cannot be regarded as absolute evidence that the symptoms produced are not due to a release of this substance since, when the symptoms of histamine intoxication produced in patients by the subcutaneous injection of histamine are at their height, not only is the blood histamine not increased but it may even be diminished (Rose, 1940).

The decrease of the blood histamine following wheal formation is perhaps less difficult to interpret if it is believed that histamine is carried for the most part by the eosinophiles (Code, 1937). Since it has been shown that there is a gradual increase of the eosinophiles in the tissues of a wheal produced by the injection of an antigen into the skin of an allergic patient, or in those of one produced by the injection of histamine, and that this increase reaches its maximum in approximately thirty minutes (Kline, Cohen, and Rudolph, 1932), it may be that the histamine is carried into the wheal by this mechanism, thereby producing a decrease in the blood histamine. This histamine also may be responsible in part for the maintenance of the wheal. This is further supported by the findings to be reported in a subsequent communication showing that the histamine content of the blood of patients with angioneurotic edema is at a very low level during an attack as compared to the level found during the quiescent phase (Rose, 1940). Another possible explanation of this decrease is an excretion of histamine into the gastric secretion, which is discussed elsewhere (Rose and Browne, 1940).

The observations of Abramson, Engel, Lubkin, and Ochs (1938) are interesting in this connection. They do not believe that histamine is responsible for wheal production, on the basis of a failure to remove histamine from allergic wheals by reversed iontophoresis, whereas this may be done from histamine wheals. It may be that, when histamine is injected or transferred through the skin by iontophoresis, a certain residual amount is always available for removal by reversed iontophoresis, whereas relatively small amounts may be released by an antigen-antibody reaction in the skin of a sensitive person without an excess of histamine being present in a free form, although it is probable that relatively larger amounts may be present in the eosinophiles.

The results of the present experiments and the decrease of blood histamine observed during surgical shock (Rose and Browne, 1940),

acute angioneurotic edema (Rose, 1940), and at the height of symptoms produced by subcutaneous injection of histamine indicate that symptoms of histamine intoxication may be accompanied by a lowered blood histamine level. The mechanism of this decrease and its relation to the production of symptoms are as yet obscure.

CONCLUSIONS

In fifteen cases of sensitivity, including ten cases of dermatographia, three of cold allergy, and one each of heat and photosensitivity, the blood histamine was studied at intervals after stimulation of the skin.

An increase of the blood histamine occurring within fifteen minutes after the stimulation was observed in eight cases. In four instances, a secondary decrease was observed occurring at or about the thirty-minute interval. In two additional cases a decrease in the blood histamine was noted following the production of wheals by oral administration of sulfamethylthiazole and by the intracutaneous injection of a drug preparation.

The observations in these and other experiments make it probable that symptoms of histamine intoxication are associated with a decrease in the blood histamine rather than an increase of this substance in the blood.

The author wishes to thank Dr. J. S. L. Browne for his valuable criticism and helpful suggestions. Thanks are also due Dr. A. H. Henderson and the Department of Allergy of the Royal Victoria Hospital for their kindness in providing many of the patients, and to Mrs. E. V. Harkness for technical assistance.

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HISTAMINASE IN ALLERGY: A STUDY OF ITS EFFECTS ON SKIN REACTIVITY TO HISTAMINE AND TO ALLERGENS

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THE theory that histamine is the important mediator in anaphylaxis has been adequately proved for the common species of experimental animals.[1-8] Because of the many analogies between human allergy and animal anaphylaxis there is a strong presumption that the symptoms of allergic disease are also mediated by histamine, liberated by sensitized tissues after contact with the specific allergen or allergens. Moreover, as Lewis[9] has shown, there exists a close parallelism between the manifestations of allergic skin reactions and those of skin reactions to histamine solutions.

Histamine is destroyed in the test tube by an enzyme, histaminase,[10] which is widely distributed in normal tissues. Consequently it has been surmised that the administration of preparations containing histaminase might alleviate the symptoms of allergy, and such a preparation (Torantil-Winthrop) has recently been offered commercially for this purpose. This and analogous products have been tried clinically in several disease states. Most of the results reported for allergic diseases are given in Table I. It may be seen that in urticaria and hay fever only have there been trials on large numbers of patients and that, in general, the reports are contradictory. It seems probable that the evident difficulty of arriving at a satisfactory conclusion regarding the clinical value of histaminase preparations is closely associated with the vagaries of the ordinary course of allergy.

It would obviously be desirable to be able to decide more definitely whether the available histaminase preparations exert an appreciable influence on either histamine or allergic reactivity in man. It appeared possible that the greater consistency of skin reactions together with the fact that they can be performed so as to yield reliable data for statistical analysis might offer a solution to the problem.*

We have examined the effect of histaminase preparations given orally and by injection on (1) skin reactions to histamine solutions, (2) skin reactions by the passive transfer technique, and (3) skin reactions by direct testing with an allergen on one allergic person. We have also

From the Laboratories of Dr. George Piness and Dr. Hyman Miller.

*Keeney[28] has recently reported that skin reactions to histamine are unaffected by histaminase given orally. However, no data were offered, and no attempt was made to decide whether the variations in reactions obtained were within the normal range.

TABLE I

SUMMARY OF CLINICAL TRIALS OF HISTAMINASE IN ALLERGIC CONDITIONS

ALLERGIC CONDITION	WORKERS	ROUTE OF ADMINISTRATION*	RESULTS (NO. OF CASES)		
			BENEFITED	DOUBTFUL	NO RELIEF
Asthma	Ereklentz and Ereklentz[11]	o	1		
	Moldenschardt[12]	o, p	?	?	?
	Kolbye[16]	o	1		
	Vaisberg[22]	p	1		
	Eustis[26]	o	4		
	Forman[27]	o	3		1
	Miller and Piness[29]	o			5
Hay Fever	Ereklentz and Ereklentz[11]	o	1		
	Ertl[17]	l, p	12	9	2
	Vaisberg[22]	p	1		
	Forman[27]	o	3		1
	Keeney[28]	o			15
	Miller and Piness[29]	o			3
	Prickman and co-workers[30]	o	9	4	13
Urticaria	Laymon and Cumming[20]	o, p	10	2	5
	Altose[24]	o, p	4	5	1
	Forman[27]	o	10		1
	Miller and Piness[29]	o, p		7	22
Allergic Dermatitis	Moldenschardt[12]	p	1		
	Felix[15]	o	4		
	Laymon and Cumming[20]	o, p			8
	Altose[24]	o, p		1	3
	Forman[27]	o	5		1
	Miller and Piness[29]	o, p			5
Angioneurotic Edema	Foshay[18]	?	1		
	Altose[24]	o, p	4		
	Forman[27]	o, p	3		
Physical Allergy	Roth and Horton[13]	o	1		
	Bernstein[14]	?			1
	Vaisberg[22]	p	2		
	Abel, Siebert, and Earp[23]	o	?	?	?
	Baker[25]	o	24		
Insulin Allergy	Roth and Rynearson[21]	o	1	11	
Serum Disease	Moldenschardt[12]	p	1		
	Foshay and Hagebusch[19]	o, p	24	1	3

*o, oral; p, parenteral; l, local.

†Treated by a combination of physical therapy and histaminase.

tried their effect on skin reactions by passive transfer to antigen administered by mouth or by injection at a remote site.

METHODS

General Procedure.—Repeated intradermal tests with serial dilutions of the reaction-producing substances, viz., histamine, cod protein, and Bermuda grass pollen, were made on each test subject before and after

the administration of histaminase.* Standard statistical methods[31] were used to evaluate the significance of any changes found.

The histaminase used orally was in the form of enteric coated capsules, labeled to contain 5 units† each of the enzyme. The capsules were from two lots of material received only a few days before they were used. Samples of the larger lot, which constituted more than three-fourths of the doses taken orally, were still available seven months later when an opportunity arose to have them re-assayed by the manufacturer. They were found to have retained 77 per cent of their labeled potency. It therefore seems probable that they had substantially their labeled potency when freshly used for the experiments.

The manufacturers further reported that the coating on these samples was too hard and had cracked off in some places; this would have exposed their contents to the action of stomach acid and enzymes. However, in other bottles of this lot, retained by us, the capsules were still intact, and we concluded they had been roughly handled during the return shipment.

The histaminase solution used for injection contained 1.5 units in 2.0 ml. and was made by dissolving the contents of two ampules of dried material (labeled 1.0 unit, re-assayed on parallel ampules soon after use and found to have 75 per cent of the labeled value) in 2.0 ml. of the saline diluent provided with them.

Allergen solutions were made by our usual techniques[32] and were diluted in 0.85 per cent NaCl solution containing 1:50,000 phenylmercuric acetate as preservative. Stock solutions were sterilized by Seitz filtration and then stored cold. Preliminary experiments showed that even the highest dilutions were fairly stable; nevertheless, fresh dilutions were made up every day or two throughout the work. The dilutions used differed in concentration by factors of 3.16 or 10.0. In the tables the concentrations are expressed in terms of their negative common logarithms; thus 4.0 corresponds to a 1:10,000 dilution. The concentrations of the histamine dihydrochloride solutions used are calculated as grams per milliliter of histamine base. Allergen extract concentrations are in terms of grams of dry material extracted per milliliter of solution.

Passive transfer sites were prepared by intradermal injection of 0.05 to 0.1 ml. of a 1:10 dilution of a cod reagin-containing serum, one to four days before testing.

The test injections, of approximately 0.01 ml. volume, were made intradermally, using short-beveled 26 gauge needles. The sites used were the upper outer arm, the hairless area of the forearm above the wrist, and the upper part of the back. A single skillful technician

*Supplied by the Winthrop Chemical Company as their preparation T-360.

†A unit of histaminase is defined by the manufacturers as the amount which will inactivate 1 mg. of histamine monohydrochloride during incubation at 37.5° C. for twenty-four hours.

gave all the injections on any one subject throughout the series of tests, in order to minimize this important source of variability as far as possible.

Reactions were read twenty minutes after injection by measuring the diameters of both wheal and flare with a transparent rule divided into millimeters. When the reactions were not circular, the average of the longest and shortest diameter was taken. Wheals with pseudopods were rare, owing to the dilute solutions employed. When encountered, the pseudopods were ignored. Confluent flares were measured in the direction perpendicular to a line joining the centers of the two confluent reactions. They were avoided as far as possible by proper spacing of the reactions.

The results of individual tests were certainly without significance in deciding whether the histaminase treatments produced a real effect on skin reactivity, except as they enabled us to estimate the variability inherent in the tests and consequently the reliability of the averages. For this reason and to avoid extensive tabulation each tabulated figure is the average of all the measurements made under the one set of circumstances.

Considerable preliminary testing was done to determine the influence on reaction diameters of the site selected for testing and of the previous day's testing in the same area. It was anticipated that these factors would require elaborate precautions and entail considerable difficulty in interpretation of results, since the reported results of such experiments, as summarized by Bowman,[33] have indicated that these effects are important. However, no significant influence of these factors on either wheal or flare could be detected. What small differences appeared were irregular and did not encourage the idea that significant differences might be discovered by more extensive testing. For example, the test of the influence of site involved measurements at previously unused sites of fifty reactions within the size range considered suitable (wheals 5 to 15 mm. diameter; flares 15 to 50 mm. diameter). A difference of 4.5 mm. in average flare diameter would have been considered significant. The actual differences found between the two ends of the upper arm area at two different histamine concentrations were plus 1.3 mm. and minus 3.2 mm. Accordingly, sites were sometimes re-used after twenty-four hours (never sooner), and results are reported without reference to site placement or history.

EXPERIMENTAL RESULTS

For convenience in tabulation and examination the first experiments are subdivided as follows:

Experiment 1.—The effect of oral administration of histaminase on the skin reactions to serial dilutions of histamine (Table II).

TABLE II

HISTAMINE SKIN TESTS BEFORE AND AFTER ORAL ADMINISTRATION OF HISTAMINASE

(Average of Repeated Tests. Diameters of Wheal and Flare Are Given in Millimeters.)

SUBJECT	NO. OF TESTS	−LOG OF HISTAMINE CONCENTRATION							SALINE CONTROL
		4.0	5.0	5.5	6.0	6.5	7.0	8.0	
Before Administration of Histaminase									
H. M.	1	9.0-55.0	7.0-20.0	6.0-16.0	6.0-15.0	5.0- 0.0	5.0-0.0	3.0-0.0	3.0-0.0
R. H.	8	8.4-30.2	6.4-18.1	5.1-11.9	5.0- 7.5	4.4- 1.2	3.1-0.0	3.0-0.0	2.6-0.0
W. S.	6	9.7-48.3	6.3-37.5	5.7-30.0	5.5-24.2	4.3-14.2	3.2-0.0	3.5-0.0	3.5-2.0
M. K.	1	9.0-55.0	7.0-30.0	6.0-20.0	5.0-15.0	5.0-10.0	4.0-0.0	3.0-0.0	1.0-0.0
Weighted av.*		9.0-42.7	6.5-27.3	5.5-20.4	5.3-15.8	4.5- 7.3	3.6-0.0	3.2-0.0	2.9-0.8
After Administration of Histaminase									
H. M.	2	10.0-37.5	7.5-27.5	6.0-15.0	5.0- 0.0	3.5- 0.0	3.0-0.0	3.0-0.0	
R. H.	4	6.5-35.0	6.2-20.0	5.7-13.0	5.7- 9.0	4.0- 1.8	3.0-0.0	3.5-0.0	
W. S.	6	8.0-47.5	6.5-35.8	5.5-26.7	5.0-17.2	4.3- 9.0	3.3-0.0	3.0-0.0	
M. K.	3	10.0-45.0	7.3-33.3	6.0-21.7	5.3-15.0	4.7- 6.7	4.0-0.0	2.7-0.0	
Weighted av.*		7.9-41.4	6.6-28.6	5.7-19.6	5.3-12.1	4.2- 5.0	3.3-0.0	3.2-0.0	
Difference Between Weighted Averages (Before Minus After)									
Wheal		+1.1	−0.1	−0.2	0.0	+0.3			
Flare		+1.3	−1.3	+0.8	+3.7	+2.3			
Two Weeks After Administration of Histaminase									
R. H.	6	9.0-27.5	7.2-21.7		4.3- 7.5		2.8-0.0	2.7-0.0	

*Obtained by multiplying the values for each person by the number of tests done on him (both before and after), adding, and dividing by the sum of the weighting factors.

Experiment 2.—The effect of parenteral administration of histaminase on such reactions (Table III).

TABLE III

HISTAMINE SKIN TESTS BEFORE AND AFTER PARENTERAL ADMINISTRATION OF HISTAMINASE

(Average Diameter: Wheal-Flare in Millimeters)

SUBJECT	NO. OF TESTS	−LOG OF HISTAMINE CONCENTRATION					SALINE CONTROL
		4.0	5.0	6.0	7.0	8.0	
Before Administration of Histaminase							
H. M.	8	12.0-37.0	8.0-26.1	5.6-13.2	3.0-0.0	1.6-0.0	1.2-0.0
R. H.	8	11.9-27.8	8.0-17.9	6.0- 3.4	1.8-0.5	2.0-0.0	2.0-0.0
M. K.	10	10.5-39.7	8.0-32.9	5.7-20.4	2.7-0.5	1.4-0.0	2.0-0.0
Av.		11.5-34.8	8.0-25.6	5.8-12.3	2.5-0.3	1.7-0.0	1.7-0.0
After Administration of Histaminase							
H. M.	4	11.0-33.2	7.8-24.5	6.0- 9.8	4.0-0.0	2.0-0.0	
R. H.	4	10.0-31.2	6.0-17.2	3.8- 3.5	1.8-0.0	1.5-0.0	
M. K.	4	10.8-43.5	7.5-31.8	4.8- 7.8	3.0-1.0	2.0-0.8	
Av.		10.6-36.0	7.1-24.5	4.9- 7.0	2.9-0.3	1.8-0.3	
Difference Between Averages (Before Minus After)							
Wheal		+0.9	+0.9	+0.9	−0.4		
Flare		−1.2	+1.1	+5.3			

Experiment 3.—The effect of oral administration of histaminase on skin reactions to dilutions of cod protein extract in passively sensitized sites (Table IV).

TABLE IV

PASSIVE TRANSFER SKIN TESTS BEFORE AND AFTER ORAL ADMINISTRATION OF HISTAMINASE

(Average Diameter: Wheal-Flare in Millimeters)

SUBJECT	NO. OF TESTS	−LOG OF ANTIGEN DILUTION					
		6.0	6.5	7.0	7.5	8.0	8.5
Before Administration of Histaminase							
H. M.	1		6.0-30.0	6.0-20.0	7.0-15.0	4.0- 8.0	3.0- 0.0
R. H.	1	8.0-35.0	3.0-15.0		4.0- 0.0		3.0- 0.0
	2			4.5-11.0		3.5- 0.0	
M. K.	1	6.0-25.0	5.0-35.0		4.0-25.0		4.0- 0.0
	2			4.5-25.0		4.0-14.0	
Weighted av.*				5.0-18.7	5.1-12.5	3.8- 6.9	3.3- 0.0
After Administration of Histaminase							
H. M.	4	8.5-31.2		5.8-27.5	5.5-17.0	3.8- 3.8	3.0- 0.0
R. H.	3	6.3-26.7		5.3-20.0			
	4				4.8-15.5	3.2- 0.0	2.3- 0.0
M. K.	3	7.0-43.3		6.7-35.0	6.7-33.3	5.3-26.7	4.0-16.7
Weighted av.*				5.9-27.5	5.6-21.1	4.1- 9.6	3.0- 4.8

*See Table I.

Experiment 4.—The effect of parenteral administration of histaminase on such reactions (Table V).

TABLE V

PASSIVE TRANSFER SKIN TESTS BEFORE AND AFTER PARENTERAL ADMINISTRATION OF HISTAMINASE

(Average Diameter: Wheal-Flare in Millimeters)

SUBJECT	NO. OF TESTS	−LOG OF ANTIGEN DILUTION				
		4.0	5.0	6.0	7.0	8.0
Before Administration of Histaminase						
H. M.	2	11.0-36.0	8.0-25.0	4.0-16.0	3.0-9.0	3.0-0.0
R. H.	2	12.0-40.0	9.0-28.0	6.0-18.0	4.0-6.0	3.0-0.0
M. K.	2	10.0-43.0	8.0-42.0	8.0-29.0	4.0-22.0	2.0-7.0
Av.		11.0-40.0	8.0-32.0	6.0-21.0	4.0-12.0	3.0-2.0
After Administration of Histaminase						
H. M.	2	9.0-34.0	8.0-30.0	3.0-8.0	2.0-2.0	2.0-0.0
R. H.	2	10.0-35.0	9.0-28.0	3.0-18.0	3.0-2.0	2.0-0.0
M. K.	2	10.0-36.0	7.0-32.0	6.0-34.0	3.0-17.0	2.0-0.0
Av.		10.0-35.0	8.0-30.0	4.0-20.0	3.0-7.0	2.0-0.0

Experiment 5.—The effect of oral administration of histaminase on skin reactions to dilutions of a Bermuda grass pollen extract in an allergic person (hay fever) (Table VI).

Experiment 6.—The effect of parenteral administration of histaminase on such reactions (Table VII).

TABLE VI

DIRECT SKIN TESTS BEFORE AND AFTER ORAL ADMINISTRATION OF HISTAMINASE

(Average Diameter: Wheal-Flare in Millimeters)

SUBJECT	NO. OF TESTS	−LOG OF ANTIGEN DILUTION					
		5.0	5.5	6.0	6.5	7.0	8.0
Before Administration of Histaminase							
R. H.	3	6.0-32.0	6.3-25.0				
	8			4.6-19.0	4.4-12.0	3.3-1.2	3.4-0.0
After Administration of Histaminase							
R. H.	5	5.6-28.0				2.4-0.0	1.8-0.0
	6		5.5-18.0	5.0-10.0	3.8-4.3		
Two Weeks After Administration of Histaminase							
R. H.	6	7.5-24.0		5.2-14.0		3.8-3.2	2.5-0.0

TABLE VII

DIRECT SKIN TESTS BEFORE AND AFTER PARENTERAL ADMINISTRATION OF HISTAMINASE

(Average Diameter: Wheal-Flare in Millimeters)

SUBJECT	NO. OF TESTS	−LOG OF ANTIGEN DILUTION			
		5.0	6.0	7.0	8.0
Before Administration of Histaminase					
R. H.	8	10.6-26.0	8.0-20.0	5.6-9.5	2.4-0.0
After Administration of Histaminase					
R. H.	4	8.6-26.2	5.8-14.2	2.2-6.8	1.5-0.8

All of the odd numbered experiments (oral administration of histaminase) were done simultaneously. During a preliminary period of two weeks repeated tests were done to measure normal reactivity. Three days after the conclusion of the preliminary tests, doses of four capsules of histaminase were taken as follows: first day at 12 noon and again at 5 P.M.; second day at 7 A.M., 10 A.M., 1 P.M., 5 P.M., and 10 P.M.; third day at 8:30 A.M. The total dose of histaminase was 160 units per person. In an effort not to miss a possible peak of activity the test sets were put on 3.5, 8.5, and 25.5 hours after the last dose of histaminase on M. K. and R. H., and at 3.5 and 25.5 hours on H. M. and W. S. In some cases they were repeated several times at a single sitting. No difference between the different time intervals appeared, so the results were lumped together for presentation. As indicated in the tables, histamine and Bermuda grass tests were again repeated on R. H. after two weeks more had elapsed.

Four months after the completion of the experiments on the effect of oral administration of histaminase a supply of histaminase for injection became available and the experiments with parenteral administration of histaminase were done at that time. Preliminary testing was done during the five days preceding the histaminase administration.

Each person was then injected intramuscularly with histaminase (1.5 units in 2.0 ml.). One and two hours later the skin tests were repeated. Again no difference could be seen between the post-treatment time intervals, and they were therefore lumped together.

It will be noted that the reactions by the passive transfer technique were considerably smaller at this time than in the earlier experiments. It is probable that the change was due to deterioration of the reagin serum during the four-month interval. It was stored on ice as the sterile 1:10 dilution in saline which was prepared freshly for Experiment 3.

Statistical tests[31] of the significance of the differences in averages before and after the histaminase treatments in all of these experiments showed that any of them could readily have resulted from the variations inherent in the testing technique and other uncontrolled factors. The greatest indication of significance was found in Experiments 5 and 6 at the higher antigen dilutions. Even here it would be quite unsafe to conclude that a real effect of the preparations on the skin reactions had been observed.

The histamine solution tests were the most extensive and were, in addition, slightly less variable than the allergen tests. Consequently they might be expected to provide the most sensitive index of histaminase action. Furthermore, it is difficult to conceive of a histaminase action on allergic skin reactions in the absence of an effect on histamine skin reactions if the theory that allergic reactions are mediated by histamine is correct. Since this theory forms the basis for the use of histaminase in allergy, it is probable that, if there were a slight but real effect on the Bermuda grass reactions, it should in any event be attributed to some nonspecific action of the preparations. In addition, slight effects due to site exhaustion, etc., were not ruled out by the less extensive preliminary testing which was reported in the introduction. If a definitely significant effect had been obtained in any experiment, it would have been necessary to provide more elaborate controls to prove convincingly that it was actually caused by the histaminase activity of the preparations.

It is of interest in passing to note that our measurements indicate that there is a linear relationship between the logarithm of concentration and the reaction diameters, for both flare and wheal, in contrast to the hyperbolic relation reported by von Gröer[34] for the wheals produced by a variety of irritants, including histamine. However, we did not attempt to produce and measure large wheals, and our measurement technique differed somewhat from his, so we hesitate to conclude that our data contradict his.

In considering how to view our findings in terms of a possible action of histaminase preparations on the clinical course of allergic disease, it occurred to one of us (H. M.) that inasmuch as we have, in passive transfer reactions which are set off by remote administration of the

allergen, a reaction which is theoretically analogous to the normal mechanism of allergic urticaria, a disease in which histaminase has been reported to be of value (Table I), it would be desirable to examine the effect of histaminase on this type of skin reaction. Accordingly, we have carried out two further experiments.

Experiment 7.—The effect of oral administration of histaminase on the reactions of local passive transfer sites to ingested allergen.

Only one of us (H. M.) proved suitable for the experiment, using the same fish-reagin serum as before (the serum produced sites sensitive to several species of fish). A number of skin sites were sensitized; a twenty-four-hour interval was allowed to elapse; then a generous helping of kippered herring was eaten. Within fifteen minutes all sites showed marked reactions with wheals approximately 20 mm. and flares 60 mm. in diameter. One week later similar passive transfer sites were prepared. Twenty-four hours later five capsules of histaminase (25 units) were taken. Three hours later 25 units were taken; then one 5-unit capsule was taken every hour for three hours; and another 25 units were taken at the end of four hours (90 units in all). After another one-hour interval a generous helping of fish was eaten. Within fifteen minutes the passive transfer sites reacted as before. Several repetitions of these tests gave the same results. No attempt was made to quantitate these results, but certainly no marked change of sensitivity consequent to taking the histaminase was seen.

Experiment 8.—The effect of oral administration of histaminase on the reactions of local passive transfer sites to remotely injected allergen.

Experiment 7 was repeated, with the exception that the test dose of antigen was 1.0 ml. of a 1:1,000 cod protein extract administered in the opposite arm. Again the prepared sites reacted similarly before and after the histaminase was taken.

COMMENT

The theory that histamine or a histamine-like substance that is destroyed in vitro by histaminase mediates allergic phenomena has led to the therapeutic trial of histaminase in clinical allergy. Through the use of the wheal and flare of the intradermal skin test as a typical allergic phenomenon subject to fairly exact control and measurement, the present investigation attempted to demonstrate an in vivo activity of histaminase which might furnish an experimental basis for its clinical use in the prevention and alleviation of allergic symptoms. No activity could be demonstrated by the method used, even though pains were taken to provide conditions most favorable for the demonstration.

CONCLUSIONS

1. Oral and parenteral administrations of histaminase preparations did not significantly influence skin reactions to histamine dilutions or the sensitivity of passively sensitized sites to a fish allergen.

2. The histaminase preparations may have slightly affected direct skin sensitivity to a grass allergen in an allergic individual, but the results could have been accidental. Furthermore, theoretical considerations suggest that, if the effect is real, it is probably not due to a specific action of the histaminase contained in the preparations.

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THE INFLUENCE OF HISTAMINASE ON THE COURSE OF ANAPHYLAXIS AND HISTAMINE SHOCK IN GUINEA PIGS

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IT APPEARS on the basis of present knowledge that anaphylaxis, anaphylactoid phenomena, nitritoid crises, and histamine shock, although physiologically similar in many respects, in regard to end points, differ so basically as to causative factors or agents that the question arises as to the primary or secondary importance of causes and effects.

Histamine shock most closely approximates the picture of true anaphylaxis as indicated by Dale and Laidlaw[13] and Wells.[39] The two conditions differ in certain important respects. Histamine reactions do not require sensitization; shocklike reactions to histamine recur uniformly on repeated injections, i.e., no desensitization occurs; and temperature reactions as well as blood coagulability changes characteristic of anaphylaxis fail to occur.

Certain other important differences between anaphylaxis, anaphylactoid phenomena, and histamine shock are demonstrable through drug antagonisms, as shown by Brolin and Saint-Girons (1920) and others.[3, 14, 15, 24, 26, 27, 29, 33, 39]

Histamine may be derived by enzymic action or bacterial action on histidine, and this, as well as other amines, are among the products of normal metabolism. Consequently this compound, or a closely related substance, is a normal constituent of all tissues.[12] The values present in various organs and body fluids have been reported by Best and McHenry.[6] A similar extensive study has been reported by Zeller and his co-workers.[43]

The physiologic significance of histamine values and its relation to anaphylaxis have been reported by Watanabe,[38] Hosoya and Watanabe,[28] and Went and Lissak.[41] Barsoum and Gaddum[4] and Anrep and his associates[2] have indicated that histamine is a normal constituent of venous blood following cardiac or skeletal muscle contraction and the values present parallel the work done.

Dragstedt and Gebauer[17] demonstrated the presence of histamine in the blood of the dog during anaphylaxis. In further work, Dragstedt and Mead[18] reported, "These observations demonstrate that the circulatory reaction and death in dogs due to anaphylaxis is completely due to histamine and that histamine intoxication is the most important feature of the reaction. The intoxication does not explain changes in blood coagulation, loss of complement, etc., but these changes are evidently

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not greatly concerned in determining the immediate issue of death or recovery.'' Dragstedt and co-workers,[17-20] Code,[7-10] Unger and Bolgert,[37] Jager,[30] Katz,[32] and others have in general confirmed and extended these observations on other species.

The mechanism involved by means of which the body detoxifies such a pharmacologically active product of metabolism was first suggested by Eustis,[22] who reported that liver of the common buzzard *(Cathartes aura)* was capable of detoxifying solutions of histamine. Best[5] also demonstrated that the body protective mechanism was mediated by means of a substance having all the physical characteristics of an enzyme. This preparation, which occurred in variable quantities in all tissues tested, was found to inactivate or destroy blood histamine. Histaminase or diamino-oxidase has likewise been shown to neutralize the effects of putrescine, cadaverine, agmatine, spermine, and spermidine.[42]

Felix[23] was unable to modify anaphylactic responses to provocative doses of an antigen by a short pretreatment with histaminase. Corper and Cohn[11] also were unable to influence the intoxication of tuberculo-anaphylaxis and tuberculo-allergy by means of histaminase. However, Karady and Browne[31] reported that 3 units of histaminase by vein fifteen minutes before a normally lethal intraperitoneal dose of histamine saved 90 per cent of the treated guinea pigs. Corper and Cohn and Alexander and Bottom were unable to confirm these observations.

Alexander[1] observed that the histamine content of the blood of rabbits following the vein injection of 8 to 10 units of histaminase remained unchanged for thirty to 120 minutes but rapidly dropped thereafter and remained at practically zero for two to four hours, after which the values gradually increased to normal levels by the sixth to eighth hours.

The following study illustrates the effect of a potent preparation of histaminase on the course of histamine poisoning and anaphylactic shock in the guinea pig.

METHODS

The toxicity of histamine base administered either subcutaneously or intraperitoneally as a saline solution of the dihydrochloride salt was determined for normal adult guinea pigs. Initial tests included a study of the influence of a short period of pretreatment of guinea pigs (fifteen to 120 minutes before the shock dose of histamine) with histaminase on usual histamine reactions when administered in antidotal doses (as indicated by incubation tests) exactly corresponding to or exceeding the final lethal shock dose of histamine. Initial tests included a study of the influence of histaminase on the course of fatal histamine poisoning.

Histaminase was injected in a range of doses by different routes at time intervals ranging from zero to 180 minutes preceding a lethal intraperitoneal dose of histamine. Additional groups of ten or more animals were then treated either by attempts at desensitization with histamine or by premedication with histamine either subcutaneously

at intervals or orally in the diet over a period of twenty-one days with or without intravenous doses of histaminase fifteen to 180 minutes immediately preceding the predetermined lethal (100 per cent) shock dose of histamine.

In the second study groups of normal guinea pigs weighing 350 to 450 Gm. were segregated and maintained on either (1) a normal balanced breeding diet or (2) the same breeding diet in which a standardized histaminase powder had been incorporated in a ratio of 9 parts of diet and 1 part of histaminase powder. These diets were fed for seven days before sensitization and for twenty-one days after sensitization. All guinea pigs were shocked with a provocative dose of the antigen twenty-one days after sensitization. A third group, also on the breeding diet, received histaminase subcutaneously in 5 unit doses every third day from the fifth to twenty-first day postsensitization with either histaminase, horse serum, or hog serum.

Materials Used.—The histamine dihydrochloride was obtained from the Eastman Kodak Company, Special Chemicals Division. Solutions were prepared freshly within one hour of use. All doses were calculated as the base.

The histaminase powder incorporated in the diet was obtained from the commercial Torantil stock powder prepared from edible animal kidneys. This material assayed 15 mg. to the unit, i.e., that quantity which on incubation with a histamine salt in a buffered menstruum (pH 7.4) for twenty-four hours at 37.5° C. inactivated 1 mg. of histamine hydrochloride. The histaminase injected parenterally was water soluble. One unit was represented by 1.2 to 2 mg. of the original powder.

RESULTS

The maximum tolerated intraperitoneal dose of histamine base (injected as a soluble salt) for the unanesthetized guinea pig weighing 250 to 350 Gm. is less than 2.5 mg. per kilogram. This dosage killed one of a small series of five pigs. A dosage of 4 mg. per kilogram killed all of twelve pigs, and a dosage of 5 mg. per kilogram killed all of seventeen pigs (Table I). Karady and Browne[31] observed that an intraperitoneal dosage of 4 mg. of histamine base as a soluble salt killed only seven of ten guinea pigs weighing 200 to 250 Gm.

Histamine Desensitization.—In order to determine whether lethal responses to histamine could be influenced by chronic medication with histamine, a tolerated but mildly toxic dosage (100 gamma) of histamine was injected daily subcutaneously for a period of ten days. We observed that a subcutaneous dosage of 0.5 mg. per kilogram killed a small percentage of a series of guinea pigs. The data of Table I indicate that under the conditions of test attempts at desensitization with histamine at intervals over a ten-day period did not influence the lethal effects of a normally fatal intraperitoneal dose of histamine.

TABLE I

THE INFLUENCE OF ORALLY AND PARENTERALLY ADMINISTERED HISTAMINASE ON HISTAMINE SHOCK IN THE GUINEA PIG

NO. OF G. PIGS PER SERIES	WEIGHT RANGE (GM.)	PRETREATMENT	HISTAMINASE DOSE INJECTED (UNITS)	HISTAMINE AS HCl SALT			PER CENT OF SURVIVALS	TIME OF DEATH IN NONSURVIVORS (MIN.)
				BASE DOSE (MG./KG.)		MIN. AFTER HISTAMINASE INJECTION		
8	(310-800) 410			2.5	intrap.		87.5	44
5	(250-292) 272			3.25	intrap.		60.0	(38-75) 56.5
23	(240-350) 305			4.0	intrap.		0.0	(20-80) 37.5
4	(300-360) 305			3.0	subc.		0.0	(5-7) 7
11	(280-320)			1.5	subc.		81.82	More than 8 hours
7	(286-350) 326	Histamine 100 γ subcut. daily 1, 3, 5, 7, 9, 10 days		4.0	intrap.		0.0	(15-55) 42.5
10	(250-330) 302	Histaminase subc. 5 units/inj. 1, 3, 5, 7, 9, 10 days before histamine	30 subc./kg.	4.0	intrap.	180	10.0	(11-85) 28.0
10	(250-366) 300	Histaminase subc. 5 units/inj. 1, 3, 5, 7, 9, 10 days before histamine	150 subc./kg.	4.0	intrap.	60	20.0	(13-30) 17.5
10	(280-346) 280	Histaminase subc. 5 units/inj. 1, 3, 5, 7, 9, 10 days before histamine	12 vein/kg.	4.0	intrap.	30	0.0	(10-35) 15.0
15	(310-386) 330	Histaminase subc. 7.5 units/inj. 1, 3, 5, 7, 9, 10 days before histamine	17 vein/kg.	4.0	intrap.	180	20.0	(6-60) 10.0
18	(252-390) 304	Histaminase subc. 7.5 units/inj. 1, 3, 5, 7, 9, 10 days before histamine	18 vein/kg.	4.0	intrap.	30	38.8	(15-$>$240) 16.0
5	(500-710) 650	Normal saline 20 c.c. per kg. plus histamine		4.0	intrap.	0	60.0	(75-∞)
5	(350-710) 640	Histaminase	400 intrap./kg.				20.0	More than 15 hours
6	(350-800) 640	Histaminase plus histamine (mixed)	200 intrap./kg.	4.0	intrap.	0	33.3*	(40-∞) 60
10	(350-720) 630	Histaminase plus histamine (mixed)	400 intrap./kg.	4.0	intrap.	0	90.0†	(80-∞) $>$15 hours
16	(230-366) 322	Breeding diet plus 10% histaminase for 10 days preceding shock		4.0	intrap.		12.5	(16-55) 45.0
18	(290-350) 313	Breeding diet plus 10% histaminase for 10 days preceding shock	24 vein/kg.	4.0	intrap.	30	16.6	(17-60) 35.0
17	(240-650) 350			5.0	intrap.		0.0	(11-43) 22.5
6	(230-270)		6.6 vein/kg.	4.0	intrap.	15	0.0	(22-55) 28.0
12	(230-275)		6.6 vein/kg.	5.0	intrap.	15	0.0	(15-43) 18.0
12	(350-650)		6.6 vein/kg.	5.0	intrap.	30	33.3	(20-40) 25.0
10	(355-670)		6.6 vein/kg.	5.0	intrap.	75	20.0	(12-50) 25.0
18	(410-750)		6.6 vein/kg.	5.0	intrap.	150	11.2	(7-50) 21.0

Symptoms were labored respiration, convulsive movements, depression and prostration.

*Histamine deaths were less acute and delayed as compared with controls.

†Eighty per cent of series lived more than fifteen hours after histamine injections, but only 20 per cent (two of ten) survived indefinitely due to visceral irritation from the histaminase solution injected.

The influence of premedication with histaminase administered subcutaneously in doses of 5 to 10 units per guinea pig at intervals for a period of ten days before the shock dosage of histamine administered intraperitoneally was also tested on five different groups of guinea pigs.

Such therapy in a dosage of 5 units given ten, seven, five, three, one and zero days before the intraperitoneal injection of a normally lethal dosage of histamine under optimal conditions modified the severity of the shock reaction and reduced the toxicity of histamine approximately 20 per cent (Table I). The data suggest that intravenous dosages of histaminase thirty minutes before the administration of histamine intraperitoneally have a slightly greater protective action than corresponding doses given at either shorter or longer intervals preceding histamine administration. The significance of these data is open to question in that observations indicated that such treatment may exceptionally be ineffective, although deaths were delayed, and that permanent survivals occurred only in those pigs that remained inactive during the twenty- to sixty-minute period following the histamine injection, thus raising the question as to the relative importance of therapy and inactivity of the guinea pig with a resulting minimal respiratory embarrassment due to oxygen deficiency and reflex reactions due to excitement following histamine shock.

The incorporation of a potent histaminase powder in the diet of a series of sixteen guinea pigs for a period of ten days prior to shocking with histamine modified the usual reaction and saved two of a series of sixteen pigs from an otherwise fatal intraperitoneal dosage of this amine. Supplementary intravenous medication with histaminase thirty minutes before administration of histamine intraperitoneally slightly accentuated the prophylactic effects of histaminase given orally. However, the protective value of histaminase by either or both routes bore no relation to the expected antagonistic effects of the enzyme on the vasodepressor or smooth muscle actions of histamine on the basis of biologic assays after incubation of mixtures of histaminase (1 unit)—histamine HCl (1 mg.) in a buffered medium at 37.5° C. for twenty-four hours. However, when the ratio of histaminase to histamine was increased to 50 to 1, inactivation of histamine was complete within thirty minutes at 37.5° C. and within five minutes or less when the ratio was 100 to 500 to 1.

An attempt was made to reproduce under conditions in vivo in the guinea pig the antidotal effects of histaminase against absolute fatal doses of histamine (Table I).

A group of five guinea pigs was injected intraperitoneally with a physiologic saline solution (20 c.c. per kilogram) of histamine in a dose of 4 mg. per kilogram. All animals were depressed and experienced respiratory difficulties typical of histamine poisoning, but three survived indefinitely. The other two died fifty-five and seventy-five minutes after the combined injection.

A series of five guinea pigs were medicated intraperitoneally with a standardized tissue extract in a dosage corresponding to 400 units of histaminase per kilogram of body weight. This treatment was not well tolerated in that all guinea pigs were depressed and only one animal survived indefinitely. The remaining four animals died more than fifteen hours after treatment due primarily to visceral irritation.

The combined intraperitoneal injection of 10 c.c. of a tissue extract representing 200 units of histaminase per kilogram of body weight and 4 mg. of histamine per kilogram delayed the course of poisoning and reduced the toxicity of histamine materially. Similar injections, when the ratio of histaminase to histamine was increased to 100 to 1, reduced the toxicity of histamine by 90 per cent. It appears, therefore, that it is possible to antidote materially by means of histaminase the effects of a 100 per cent lethal intraperitoneal dose of histamine. Although nine of ten animals so treated survived more than fifteen hours, an indefinite survival occurred in only 20 per cent of the series due to local reactions to the extract injected. We believe that a less irritant and more potent histaminase preparation might permit a greater percentage of permanent survivals from acutely lethal doses of histamine.

The intravenous injection of histaminase in a dosage of 6.6 units fifteen minutes before dosages of 4 or 5 mg. of histamine base per kilogram of body weight did not materially influence either the normal symptomatic responses or lethal effects of histamine. Similar doses of histaminase given at longer periods preceding an otherwise lethal dose of histamine protected a small percentage of a significant number of guinea pigs. The protective effects of medication were greatest at the thirty-minute time interval and diminished as the prehistamine injection time interval was lengthened.

When all guinea pigs, which were pretreated with histaminase given orally or with oral and small parenteral doses, were grouped, a total of thirteen or 20 per cent of sixty-three guinea pigs survived an otherwise fatal dose of histamine. Of a total of forty animals treated thirty or more minutes before the intraperitoneal injection of histamine, seven, or 17 per cent, of the series survived. The observed protective values of the two methods of treatment are not significantly different and suggest that any protection afforded under the conditions of these tests was due primarily to the parenterally administered histaminase.

The circulatory or isolated organ responses to mixtures of histaminase and histamine in a ratio of 1 to 100 units of histaminase to 1 mg. of histamine hydrochloride differ in no essential respect from those of corresponding doses of histamine hydrochloride per se, if the mixture is injected by vein in anesthetized cats or added to the organ baths immediately after mixing. In this respect the effects of histaminase differ materially both qualitatively and quantitatively from similar antagonistic effects of amino acids on histamine responses as described by Edlbacher and his co-workers.[21] However, if the ratio of histaminase

to histamine is materially increased, complete destruction or inactivation of histamine can be demonstrated within thirty minutes or less. Thus, incubation at 37.5° C. of mixtures of histamine hydrochloride (1 mg.) with 5, 10, 20, 40, 50, 100, 200, or 500 units of histaminase in a buffered medium resulted in the complete inactivation of the histamine as indicated by the absence of the usual histamine vasodepressor responses in anesthetized cats within 5, 4, 3, 1½, 1, ½, ¼, and $\frac{1}{20}$ hours.

The potency of the histaminase used in the majority of these experiments was of such relatively low order that the parenteral administration to animals of quantities necessary to destroy or inactivate a lethal dose of the rapidly acting histamine in ten minutes or less was impossible. However, if large doses of histaminase are used in order to compensate for the differences in the time of reaction of histaminase and histamine, the observed antagonistic efforts appear entirely compatible with the suggestion of Best and McHenry[6] that "the mechanism responsible for the inactivation of the amine (histamine) in the body of naturally occurring or added histamine and having the characteristics of an enzyme may have considerable physiologic significance."

Karady and Browne[31] reported that treatment of guinea pigs under light ether anesthesia with histaminase given intravenously in a dosage of 3 units fifteen minutes before the intraperitoneal injection of 4 mg. of histamine base saved eighteen of twenty guinea pigs. The disproportion between the dosages of histaminase and histamine given by these authors together with our inability to confirm their observations when using unanesthetized guinea pigs raised the question as to the inhibitory influence of ether anesthesia on the histamine shock as reported by Heffter[27] and Lumiere.[34]

Anaphylaxis Following Sensitization With Histaminase or With Hog Serum.—The intravenous administration of a provocative or potent dose of kidney powder on the twenty-first day after sensitization with this antigen caused fatal anaphylactic reactions in an increasing percentage of the shocked pigs up to a dosage of 2 units. This dosage killed 50 per cent of a series of ten animals. The responses to larger provocative doses of histaminase were of mixed type, i.e., bronchiolar and circulatory in that survival periods were longer and circulatory depression was more prominent.

The administration of a provocative dosage of 0.25 c.c. of hog or horse serum was uniformly lethal (Table II). An intravenous dose of 2 units of histaminase following sensitization with hog serum caused slight to moderate symptoms, but no fatalities. Under the conditions of the test, sensitization with hog serum does not sensitize to histaminase from the same animal source. The data suggest that the treatment of a series of hog serum-sensitized guinea pigs with histaminase administered subcutaneously over a period of ten days with or without supplementary injection by vein ninety minutes before shocking with hog

TABLE II

COMPARISON OF HISTAMINASE, HOG SERUM, AND HORSE SERUM AS ACTIVATING ANTIGENS

NO. OF ANIMALS	SENSITIZATION BY SUBCUTANEOUS INJECTION		SHOCK INTRAVENOUS INJECTION 21 DAYS AFTER SENSITIZATION		NATURE OF REACTION				TIME OF DEATH (MIN.)		PER CENT OF SURVIVALS
	AGENT	DOSE (C.C.)	AGENT	DOSE	DEPRESSION	ANAPHYLAXIS			RANGE	MEAN	
						SLIGHT	MODERATE	COMPLETE			
10	Hog serum	0.1	Histaminase	2 units	0	0	10	0			100.0
4	Hog serum	0.1	Hog serum	0.125 c.c.	0	0	2	2	1-4	2.5	50.0
8	Hog serum	0.1	Hog serum	0.25 c.c.	0	0	0	8	2-7	4.0	0.0
10	Hog serum	0.1	Hog serum	0.5 c.c.	0	0	0	10	3-18	4.0	0.0
10	None		Horse serum	0.5 c.c.	0	0	0	0	0	0	100.0
10	Horse serum	0.1	Horse serum	0.125 c.c.	0	0	5	5	2-7		50.0
10	Horse serum	0.1	Horse serum	0.25 c.c.	0	0	0	10	1-6		0.0
10	Horse serum	0.1	Horse serum	0.5 c.c.	0	0	0	10	1-12		0.0

TABLE III

EFFECT OF MULTIPLE SUBCUTANEOUS INJECTIONS AND A SINGLE INTRAVENOUS INJECTION OF HISTAMINASE ON THE COURSE OF ANAPHYLACTIC SHOCK

NO. OF ANIMALS	SENSITIZATION BY SUBCUTANEOUS INJECTION (DOSE, 0.1 C.C.)	PRETREATMENT WITH HISTAMINASE		TIME BETWEEN HISTAMINASE INJECTION AND SHOCK INJECTIONS OF ANTIGEN (MIN.)	SHOCK INTRAVENOUS INJECTION OF ANTIGEN		TIME OF DEATH† AFTER SHOCK INJECTION (MIN.)		PER CENT OF SURVIVALS
		SUBCUTANEOUSLY 5 UNITS	INTRAVENOUSLY BEFORE SHOCK (UNITS)		ANTIGEN	DOSE (C.C.)	RANGE	MEAN	
8	Hog serum	None	None		Hog serum	0.25	2-7	4.0	0.0
10	Hog serum	None	None		Hog serum	0.5	3-18	4.0	0.0
12	Hog serum	5 times*	None		Hog serum	0.25	4-7	3.5	75.0
12	Hog serum	5 times*	2	60	Hog serum	0.25	35-50	42.0	25.0
15	Hog serum	5 times*	2	150	Hog serum	0.25	13-32	22.5	66.6
10		None	None		Horse serum	0.25	1-6		0.0
10	Horse serum	None	None		Horse serum	0.5	1-12		0.0
20	Horse serum	5 times*	None		Horse serum	0.25			100.0
6	Horse serum	5 times*	0.25	50	Horse serum	0.25	6-42		33.3
6	Horse serum	5 times*	1.0	50	Horse serum	0.25			100.0
10	Horse serum	5 times*	2.0	30	Horse serum	0.25	25-32	29.0	80.0
10	Horse serum	5 times*	2.0	150	Horse serum	0.25			100.0

*At two- or three-day intervals during the second and third weeks of sensitization, doses of 5 units of histaminase were given subcutaneously to all animals except controls.

†Data refer to nonsurvivors only.

TABLE IV

EFFECT OF REPEATED ORAL ADMINISTRATION AND A SINGLE INTRAVENOUS INJECTION OF HISTAMINASE ON THE COURSE OF ANAPHYLACTIC SHOCK

NO. OF ANIMALS	SENSITIZATION BY SUBCUTANEOUS INJECTION (DOSE, 0.1 C.C.)	PRETREATMENT WITH HISTAMINASE		TIME BETWEEN INTRAVENOUS INJECTION OF HISTAMINASE AND SHOCK INJECTION OF ANTIGEN (MIN.)	SHOCK INJECTION (INTRAVENOUS)		TIME OF DEATH AFTER SHOCK INJECTION (MIN.)		PER CENT OF SURVIVALS
		ORAL DAILY DOSE (UNITS)	INTRAVENOUS DOSE 21 DAYS AFTER SENSITIZATION (UNITS)		AGENT	DOSE (C.C.)	RANGE	MEAN	
8	Hog serum	None	None		Hog serum	0.25	2-7	4.0	0.0
10	Hog serum	None	None		Hog serum	0.5	3-18	4.0	0.0
10	Hog serum	None	2.0	90	Hog serum	0.25	19-32	27.5	40.0
10	Hog serum	80-133	None		Hog serum	0.25	5-7	5.2	50.0
10	Hog serum	80-133	2.0	90	Hog serum	0.25	5-13	10.0	30.0
10	Hog serum	80-133	2.0	150	Hog serum	0.25	6-23	14.5	50.0
10	Horse serum	None	None		Horse serum	0.25	1-6		0.0
10	Horse serum	None	None		Horse serum	0.5	1-12		0.0
10	Horse serum	None	1.0	75	Horse serum	0.125	4-6	5.0	40.0
10	Horse serum	None	2.0	40	Horse serum	0.25	3-6	4.0	10.0
10	Horse serum	None	2.0	150	Horse serum	0.25	5-39	17.0	50.0
10	Horse serum	80-133	None		Horse serum	0.25	4-29		42.8
10	Horse serum	80-133	0.25	45	Horse serum	0.25	3-5	4.5	0.0
10	Horse serum	80-133	1.0	45	Horse serum	0.125	30-58	48.0	60.0
10	Horse serum	80-133	2.0	30	Horse serum	0.25	4-35	11.5	20.0
10	Horse serum	80-133	2.0	30	Horse serum	0.25	6-90	24.5	20.0
10	Horse serum	80-133	2.0	150	Horse serum	0.25	18-25		50.0

or horse serum reduced the lethal effects of this reagin and prolonged the average period of survival (Table III).

The incorporation of a potent kidney powder in the breeding diet reduced by one-half the otherwise lethal effects of a provocative dose of hog serum. The limited data do not clearly indicate whether a preliminary treatment with histaminase powder of serum-sensitized pigs before a provocative dose of serum is equivalent to or improved by combined vein and predietary treatment with the preparation (Table IV).

Parenteral medication at intervals during the last two weeks of the sensitization period significantly reduced the incidence of fatal anaphylaxis following the injection of serum. A comparison of the data of Tables III and IV suggests that the protective effects of histaminase administered parenterally are slightly superior to those of uncertain dosage following oral medication.

The intravenous injection of histaminase (1 to 2 units per guinea pig) twenty-one days after sensitization with horse serum produced no symptoms other than those due to handling. However, premedication with such doses forty to 150 minutes before shocking with an otherwise lethal dose of horse serum reduced the incidence of lethal anaphylaxis. The saving value of such treatment appeared to increase somewhat with the length of the treatment period preceding the horse serum.

The incorporation of histaminase in the diet protected 42.8 per cent of guinea pigs shocked with a lethal dose of horse serum. The additional parenteral dose of histaminase preliminary to the serum injection appeared additively beneficial.

Parenteral medication with histaminase over a two-week period with or without a premedication dose before the provocative serum greatly reduced the severity of anaphylactic symptoms and permitted the survival of a total of thirty-six of forty guinea pigs.

These data on the basis of the limited protective effects of histaminase under various conditions of treatment preceding histamine shock and the definite protection afforded against anaphylaxis when it is given preceding the injection of shock doses of either hog serum or horse serum antigens suggest that histamine shock differs materially from a physiologic standpoint from true anaphylaxis. However, the time and degree of responses of the guinea pig under the two conditions may be the determining difference.

SUMMARY

1. The usual course of symptoms and the lethal effects of unit doses of histamine was not significantly influenced by periodic medications with histamine over a ten-day period preceding the final shock dose of histamine.

2. Pretreatment of guinea pigs either orally or parenterally with a potent histaminase preparation over a ten- to fourteen-day period prior

to the administration of a normally lethal dose of histamine completely protected a small, but questionably significant, percentage of the guinea pigs.

3. The intraperitoneal injection of large volumes of saline solutions to antidote partially the effects of a lethal dose of histamine and the intraperitoneal injection of a histaminase solution containing a lethal dose of histamine in a ratio of 100 units of histaminase to 1 mg. of histamine HCl permitted the survival of 90 per cent of a series of guinea pigs more than fifteen hours. However, an indefinite survival was reduced due to local reactions to such injections.

4. On the basis of sensitization tests with hog and horse sera it appears that a fairly quantitative relation exists between the size of the provocative dose of the antigen and the course of anaphylaxis as well as the percentage of fatal reactions.

5. Under the conditions of these experiments it was possible to materially influence by means of a histaminase preparation given orally or parenterally both the course and lethal effects of otherwise absolute lethal doses of antigen administered intravenously in the guinea pig.

6. These experiments suggest that the physiologic picture of histamine shock differs materially from that of anaphylactic shock. However, on the basis of reactions contributing primarily to death, the differences may be of quantitative rather than qualitative importance.

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THE ACTION OF HISTAMINASE ON THE HISTAMINE CONTENT OF THE BLOOD OF THE RABBIT

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SINCE the demonstration by Best and McHenry[1] (1930) of a histamine-inactivating substance which they termed "histaminase," considerable literature on the subject has appeared. Much of this is clinical, and there is little information concerning the action of histaminase on histamine in the intact animal.

Atkinson and Ivy[2] prepared histaminase according to the method of Best and McHenry. When injected parenterally in doses more than sufficient to inactivate a given amount of histamine in vitro, it had no effect on the gastric secretory response produced by the same amount of histamine. These findings are not in accord with those of Suzuki,[3] who inhibited the action of histamine on gastric secretion in rabbits by giving histaminase intravenously one hour previous to histamine injection.

Guinea pigs injected with histaminase gave no symptoms of shock when histamine was administered soon thereafter, and, when sensitized, they were protected against anaphylactic shock following the parenteral injection of specific antigen.[4] These results have recently been disputed.[5]

The histaminase used in the experiments recorded here, as well as that reported in most of the recent literature, is a commercial product designated as T. 360.* Felix[6] tested this material and found that it corresponded in every respect to the properties of histaminase reported by Best and McHenry.

The quantitation of histaminase is based upon the amount of histamine inactivated by it. The procedures used to determine the quantity of histamine destroyed vary widely. The most prominent methods are the pharmacologic determination according to the technique of Barsoum and Gaddum[7] or one of its modifications, and the measurement of the fall in blood pressure after injection into atropinized animals.

The above two methods were compared to that used in experiments here reported which consisted in measuring wheal formation after electrophoresis of the histamine-containing material into normal human skin. This was found to give satisfactorily comparable results.

EXPERIMENTAL DATA

Rabbits were chosen because of the comparatively high histamine content of their circulating blood.

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*The supply of histaminase (called at first T. 360 and later Torantil) was furnished by the Department of Medical Research, Winthrop Chemical Co., Inc.

Acid histamine phosphate (Burroughs-Wellcome, U.S.P.) was used. A 1:1,000 solution of histamine (base) was prepared by adding 0.276 Gm. to 100 ml. of Tyrode's solution. Dilutions for use were made with Tyrode's solution freshly prepared.

Titration of Histaminase Preparations.—Ampoules of histaminase each containing 1 "unit"* were pooled and a known amount of histamine (base) in Tyrode's solution added to each aliquot of histaminase. The mixture was incubated at 37.5° C., and after eight and twenty-four hours it was tested for residual histamine (Protocol I).

PROTOCOL I

STANDARDIZATION OF HISTAMINASE

HISTAMINASE (UNIT)	HISTAMINE BASE		TYRODE'S SOLUTION (C.C.)	DEGREE OF SKIN WHEALING		
	(MG.)	C.C. 1:10,000 SOL.		BEFORE INCUBATION	AFTER 8 HOURS OF INCUBATION	AFTER 24 HOURS OF INCUBATION
1	0.05	0.5	9.5	++++	0	0
1	0.1	1	9	++++	0	0
1	0.2	2	8	++++	0	0
1	0.3	3	7	++++	+	0
1	0.4	4	6	++++	+++	0
1	0.5	5	5	++++	++++	+
1	0.7	7	3	++++	++++	++++
1			3	0	0	0

Result.—1 unit of histaminase (lot 7187) inactivated 0.2 mg. of histamine after eight hours and 0.4 mg. after twenty-four hours of incubation.†

Determination of Histamine or Histamine-Like Substance.—This was done by electrophoresis into human skin according to the method of Abrahamson and Gorin.[8] The apparatus consisted of a 45-volt "B" battery wired to a set of poles through a milliammeter and a rheostat. The indifferent electrode was a metal cylinder covered with blotting paper saturated with physiologic salt solution and held in the hand. The active electrode was a steel mesh grid 1 cm. square applied to the skin of the outer aspect of the arm. To this was fastened blotting paper moistened with the histamine solution to be tested. Shortly after the application of a current of 2 milliamperes for two minutes a wheal appeared. Its size was estimated fifteen minutes after the current was interrupted.

This method on most individuals will readily record dilutions of histamine base of 1:1,000,000 and gives a sharp differentiation between this dilution and those of 1:100,000 and 1:5,000,000. It was found to compare favorably with the pharmacologic method of Barsoum and Gaddum and to be consistently reliable. No attempt was made to quantitate accurately the amount of histamine in each sample of blood tested, and comparative values were recorded as ±, +, ++, and +++. Less than 1γ histamine per cubic centimeter of solution gave a ± to 0 response.

*1 "unit" (manufacturer's) of histaminase will inactivate 1 mg. of histamine hydrochloride.

†The discrepancy between the amount of histamine inactivated by 1 unit of histaminase as declared by the manufacturer and that indicated in Protocol I may be due to this method for the detection of histamine.

Determination of the Effect of Histaminase on Circulating Histamine.—Fasting rabbits were weighed, and the value of 60 c.c. of blood per kilogram of body weight used. The range of histamine in rabbit's blood has been estimated to average 10γ.[7] We assumed a maximum of 20γ per cubic centimeter. The latter figure was employed. The maximum amount of circulating histamine was thus estimated by multiplying the weight in kilograms by 60 by 20. After the quantity of histamine inactivated in vitro by a sample of the histaminase had been determined by standardization, the amount of histaminase required to inactivate all of the circulating histamine was estimated (Protocols II and III).

PROTOCOL II

EFFECT OF HISTAMINASE ON CIRCULATING HISTAMINE

TIME OF BLEEDING	DEGREE OF SKIN WHEALING		
	IMMEDIATE	AFTER 8 HOURS OF INCUBATION	AFTER 24 HOURS OF INCUBATION
Before injection	++	++	++
30 min. after injection	++	++	+
1 hour after injection	++	+	±
2 hours after injection	±	0	0
3 hours after injection	0	0	0
4 hours after injection	0	0	0
6 hours after injection	++	0	0
8 hours after injection	++	+	±
Controls			
Before injection			
2 c.c. blood +½ unit T. 360	++	0	0
2 c.c. blood +0.05 mg. histamine	+++	+++	+++
2 c.c. blood +0.05 mg. histamine + ½ unit T. 360	+++	0	0
3 hours after injection			
2 c.c. blood +0.05 mg. histamine		++	++

Rabbit A weighed 3.9 kg. Estimated maximum circulating histamine (20γ per c.c.) = 3.9 × 60 (c.c.) × 20 = 4.28 mg.

One unit T. 360 (lot 7187) was found to inactivate 0.2 mg. of histamine in eight hours (Protocol I). Therefore $\frac{4.68}{0.2}$ or 23 + units would be required for this incubation period.

Twenty-four units were injected intravenously. Blood samples were tested for histamine immediately on withdrawal and after incubation for eight and twenty-four hours.

Blood was withdrawn before injection of histaminase and at thirty minutes, one, two, three, four, six, and eight hours thereafter. This was done by cardiac puncture with a 22 gauge needle under light ether anesthesia. Each sample was tested immediately for histamine and again after eight and twenty-four hours of incubation.

Correlation Between Histamine Content and Leucocytes of the Blood.—According to Code and Ing,[9] the histamine in the circulating blood of rabbits is carried in the buffy coat, probably in the polymorphonuclear cells, the granules of which are eosinophilic. Total and differential white blood cell counts were made on each blood sample withdrawn, and these were compared to the estimated amount of histamine present in

that sample. The differential counts recorded the ratio between the granulocytes and mononuclear cells (Protocol III).

PROTOCOL III

COMPARISON OF THE NUMBER OF CIRCULATING LEUCOCYTES TO THE HISTAMINE CONTENT OF THE BLOOD

TIME OF BLEEDING	DEGREE OF SKIN WHEALING			LEUCOCYTE COUNTS		
	IMMEDIATE	AFTER 8 HOURS OF INCUBATION	AFTER 24 HOURS OF INCUBATION	TOTAL NO. (PER CU. MM.)	GRANULOCYTE	MONONUCLEARS
Before injection	++	++	++	8,800	22	78
30 min. after injection	++	++	+			
1 hour after injection	++	+	0			
2 hours after injection	±	0	0	2,950	10	90
3 hours after injection	0	0	0	1,400	12	88
4 hours after injection	0	0	0	6,250	65	35
6 hours after injection	±	±	0	7,600	77	23
8 hours after injection	++	++	±	9,800	84	16
11 hours after injection				9,500	72	28

Controls had same values as in Protocol II.

Rabbit C weighed 4.3 kg. Estimated circulating histamine = $4.3 \times 60 \times 20 = 5.16$ mg.

One unit (lot 7187) inactivated 0.2 mg. in eight hours. Therefore $\frac{5.16}{0.2}$ or 25.8 units would be required. Twenty-six units were injected as in Protocol II.

Total and differential leucocyte counts were made on samples of blood withdrawn. Granulocytes and mononuclear cells are recorded separately.

The protocols are representative. Of the twelve rabbits so treated, three showed but slight reduction of histamine following injection of the calculated dose of histaminase. The remainder showed marked reduction. In all, however, the action of histaminase was delayed for thirty minutes to two hours as indicated in the protocols.

As further controls, histaminase was heated to 80° C. for thirty minutes before injection in three rabbits, and no reduction of histamine was noted at any time.

Subcutaneous injections of histaminase were given to four rabbits, and the experiment was conducted thereafter as with the animals treated intravenously. In three there was questionable, if any, reduction of circulating histamine. In one rabbit the calculated dose of histaminase was given twice, and there was a decided diminution in circulating histamine after a delay of two hours following injection.

In almost every instance, including those in which inactivated histaminase was given, there was a prompt initial fall in the total number of leucocytes lasting three to four hours. The percentage of granulocytes, however, consistently rose to several times the initial count. These findings are attributed to the injection of a foreign protein substance.

DISCUSSION

From the experiments, it appears that histaminase given in large amounts intravenously has the power to inactivate temporarily most and

frequently all of the circulating histamine. Of interest is the fact that for a period of thirty minutes to two hours after injection, a time when the histaminase concentration of the blood presumably should be greatest, it had little or no effect whatever. This is shown when blood taken during this interval was incubated. Had histaminase been active then as in later samples, pronounced reduction of histamine should have occurred. Some three hours after injection the action of histaminase was marked even without further incubation. In a few instances this effect was noted in one-hour samples. At no time was this result obtained in vitro, wherein four to six hours of incubation seemed essential with the quantities of histaminase and histamine employed.

The reason for the initial delay in the action of histaminase is not known. Whether the enzyme leaves the circulating blood immediately or whether an antienzyme operates temporarily are problems that have not been studied. Ungar and Parrot reported that, whereas blood contains no demonstrable histaminase, a certain quantity appears immediately after bleeding and diminishes markedly after one hour. This phenomenon would scarcely explain the results recorded here. Moreover, these authors incubated blood with histamine for but two hours which, again, in our experience as well as that of Felix and others, is far too short a time for inactivation to occur in vitro.

The action of histaminase in every instance was but temporary and had essentially disappeared within eight hours.

The marked reduction of demonstrable histamine in the blood in the presence of an increased number of polymorphonuclear cells is of interest, since Code and Ing[9] have demonstrated that this substance is contained in the white blood cells. There was no correlation between the histamine content and either the total number of leucocytes or the relative number of granulocytes, which morphologically did not appear abnormal. Zon, Ceder, and Crigler[10] have recently reported that the white blood cells of the rabbit could be depressed markedly without any significant change in the blood histamine. They found indications that histamine is carried by the platelets. No platelet counts were made in the experiments here reported.

CONCLUSION

1. Histaminase injected intravenously into rabbits temporarily inactivates circulating histamine.

2. This action of histaminase is postponed for reasons unknown for thirty minutes to two hours after injection.

3. There is no correlation after the injection of histaminase between the histamine content of the blood and the total number of white blood cells or percentage of granulocytes present.

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A SLOWLY ABSORBED GELATIN-POLLEN EXTRACT FOR THE TREATMENT OF HAY FEVER

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THE disadvantages of injection therapy for hay fever are due in many instances to the too rapid absorption of the antigen. An effort has been made in this study to prepare an extract which would be more slowly absorbed. Alum-precipitated pollen extracts,[1] glycerolated extracts,[2] formalinized extracts,[3] pollen extracts in lanolin and olive oil,[4] and pollen extracts in almond oil[5] have already been attempted with this in view. Recently, gelatin has been used successfully as a medium retarding the absorption of epinephrine.[6] It is stable, nonirritating, nontoxic, and nonantigenic, properties not uniformly found in previously employed vehicles. Therefore, it seemed advisable to try a pollen extract containing gelatin. This extract was prepared by adding to one part of the standardized aqueous ragweed extract* four parts of the sterile gelatin mixture described by Spain, Strauss, and Fuchs.[6] For example, 1 c.c. of a 25,000-unit extract was added to 4 c.c. of the gelatin mixture to give a 5,000-unit gelatin-ragweed extract.

ABSORPTION TIME

The difference in the rate of absorption of the ordinary aqueous ragweed extract and the gelatin-ragweed extract was determined by passive transfer tests which were performed on eight suitable normal nonsensitive adults, according to the method devised by Cohen, Ecker, and Rudolph.[7] An area of skin on the outer aspect of the upper arm of each normal subject was passively sensitized with 0.1 c.c. of undiluted pretreatment serum from a ragweed-sensitive patient. Forty-eight hours later 1 c.c. of the aqueous ragweed extract (5,000 P.N. units) was injected subcutaneously in the opposite arm of each subject, the degree of reaction at the passive transfer site and the time elapsing until its maximum development being carefully noted.

One week later passive transfer sites were again prepared as before in the same subjects. Forty-eight hours later 1 c.c. of the gelatin-rag-

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*The ragweed pollen was defatted with ether and extracted in Coca's solution. It was standardized on the protein nitrogen unit basis, according to the method of Cooke and Stull.

weed extract (5,000 P.N. units) was injected subcutaneously in the opposite arm of each subject, and the time interval and degree of reaction at the passive transfer site were noted. The results are presented in Table I.

TABLE I

TIME AND DEGREE OF REACTION UPON PASSIVELY SENSITIZED SITE AFTER INJECTION OF RAGWEED ANTIGEN IN OPPOSITE ARM

CASE NO.	AQUEOUS EXTRACT 5,000 UNITS		GELATIN EXTRACT 5,000 UNITS	
	TIME INTERVAL (MIN.)	REACTION	TIME INTERVAL (MIN.)	REACTION
1	40	Marked	120	Moderate
2	45	Marked	140	Moderate
3	22	Marked	90	Marked
4	30	Moderate	120	Slight
5	55	Marked	180	Moderate
6	40	Marked	150	Moderate
7	25	Moderate	120	Moderate
8	45	Marked	120	Moderate

Slight, moderate erythema; moderate, small round wheal with an area of erythema; marked, large wheal, irregular, with pseudopods and an area of erythema.

The time interval between the gelatin-ragweed injection and the maximum development of the wheal at the sensitized site was increased threefold or more over the interval which elapsed after the aqueous ragweed injection. Therefore, it was possible to conclude that there was a definite decrease in the rate of absorption of the gelatin-ragweed extract.

Ninety-five adult patients suffering from ragweed hay fever symptoms were treated by injection with gelatin-ragweed pollen extract. Each patient was first tested intracutaneously, both qualitatively and quantitatively, with the aqueous ragweed pollen extracts as well as with the gelatin-ragweed pollen extracts. These tests yielded identical reactions in fifty-three patients, while in the remaining forty-two there was an increase in the size of the wheals with the gelatin-ragweed pollen extracts. This increase may have been due to the more slowly absorbed gelatin-ragweed mixture, with the consequently prolonged contact between antigen and tissue cell. Thus, a skin test developing into a "moderate" reaction with the aqueous extract appeared as a "marked" reaction with the gelatin extract, while a "marked" reaction with the aqueous extract developed into a "marked plus" reaction with the gelatin extract. The patients were classified as A, B, or C according to the degree of reactivity indicated by test with the aqueous ragweed pollen extracts;[8] A, being the most sensitive, and C, the least sensitive of the groups. This classification afforded a clue as to the average dosage schedule suitable for prophylactic treatment, which was then begun by using the gelatin-ragweed pollen extracts.

In 1939 fifty-five adult patients were treated with the gelatin-ragweed pollen extract. Eleven patients were in Class C, thirty-five in Class B,

and nine in Class A. Since the mixture was new and untried, the standard schedule of dosages usually employed with the aqueous ragweed pollen extracts (Table II) was followed. After several injections had been administered without discomfort to any patient and with only small local reactions resulting, dosages greater than those which would ordinarily be administered with the aqueous ragweed extract were given cautiously.

TABLE II

PROPHYLACTIC TREATMENT, AVERAGE DOSAGE FOR VARIOUS CLASSES

(Aqueous Pollen Extract Given in Protein Nitrogen Units)

	CLASS AA	CLASS A	CLASS B	CLASS C
Dose	1 unit	10 units	100 units	100 units
Skin reaction	Marked	Marked	Marked	Moderate
Dose	10 units	100 units	500 units	500 units
Skin reaction	Marked plus	Marked plus	Marked plus	Marked
	Units	*Units*	*Units*	*Units*
Test day	0	0	0	5
Injection 1	2	5	10	10
Injection 2	5	10	20	20
Injection 3	10	20	40	40
Injection 4	20	30	70	70
Injection 5	30	40	100	100
Injection 6	40	60	200	200
Injection 7	50*	80	300	400
Injection 8	--	100	400	700
Injection 9	--	150	600	1,000
Injection 10	--	200	800	1,250
Injection 11	--	300	1,000	1,500
Injection 12	--	400	1,250	2,000
Injection 13	--	500*	1,500	2,500
Injection 14	--	---	1,750	3,000
Injection 15	--	---	2,000	3,500
Injection 16	--	---	2,250	4,000
Injection 17	--	---	2,500*	5,000*

*This dose was given at four- to seven-day intervals during the season.

The average Class C patient acquired under treatment a tolerance for a dosage of 5,000 units of the aqueous ragweed extract. With the schedule in Table II prepared for the aqueous extract it required eighteen injections to reach this maximum, providing interruption of treatment did not demand repetition of dosage, whereas, when the gelatin-ragweed extract was used, this dose was reached in thirteen injections regardless of the lapse of treatment. Furthermore, a higher unit of tolerance was noted, an average of 7,500 units being reached easily with fourteen injections.

For Class B patients the average maximum standard dosage was 2,500 units and was reached with an average of seventeen injections of the aqueous ragweed pollen extract. The thirty-five Class B patients treated with the new extract exceeded this average maximum dosage in every case, and an average maximum dosage of 4,400 units was reached with an average of fourteen injections on treatment with the

gelatin-ragweed pollen extract. Intervals of two or more weeks between injections did not hinder an increase in dosage. While it is occasionally possible to force a greater maximum dosage when using the aqueous extract, with the gelatin extract it could usually be accomplished without fear of adverse consequences.

In the third and most sensitive group of patients, designated Class A, the usual maximum dosage level with the aqueous extract approximated 500 units, and it required thirteen injections by the old schedule to reach this dose (Table II). An average of thirteen injections was also given of the gelatin-ragweed extract, but an average top dosage of 1,500 units was reached. Some of these patients were very apprehensive of being injected because of constitutional reactions occurring during the course of previous treatments with aqueous extracts. In this group, likewise, increased dosages with the gelatin-pollen extract were possible despite irregular attendance.

Twenty-two patients who had reached maximum or near maximum dosages of the gelatin-ragweed pollen extract at the end of the hay fever season in 1939 continued their treatment on a monthly basis throughout the year 1940, the maximum dosages ranging from 2,000 to 12,000 protein nitrogen units. Although the majority of injections were administered at intervals of four weeks, a lapse of even eight weeks did not require a reduction in dosage. With the aqueous extract, such an interval is usually considered too long for reinjection except with a lessened dosage. It was noted that with the gelatin extract a change from the old to the new extract could be made directly with no untoward reactions. Some of these patients may possibly have taken much greater top doses, but no attempt was made to do so, as the ragweed concentration was often twice the amount attained with the aqueous extracts.

In 1940 the patients selected for preseasonal treatment with the gelatin extracts were those who were unable to undergo satisfactory treatment with the aqueous extracts. This group included patients who demonstrated a high degree of sensitiveness as shown by intracutaneous testing and by clinical experience. Nine patients were in Class C; fifteen, in Class B; and sixteen, in Class A. Some of the patients classified as B or C by intracutaneous tests with the aqueous extracts had demonstrated a higher degree of sensitivity than was expected when treated with these extracts and were therefore unable to reach the expected maximum dosage.

Treatment with the gelatin-ragweed mixture in 1939 had made it apparent that a schedule with a decreased number of injections was possible. Therefore the schedule employed with the aqueous ragweed pollen extracts was not followed. The Class A patient usually received

25 units of the gelatin mixture as an initial injection, and the Class B or C patient, 50 units. The schedule given in Table III was generally used.

TABLE III

PROPHYLACTIC TREATMENT, AVERAGE DOSAGE FOR VARIOUS CLASSES

(Gelatin-Pollen Extract Given in Protein Nitrogen Units*)

INJECTION	CLASS A (UNITS)	CLASS B (UNITS)	CLASS C (UNITS)
1	25	50	50
2	50	150	150
3	100	300	300
4	200	600	700
5	300	1,000	1,000
6	500	1,500	2,000
7	700	2,000	3,500
8	1,000	2,500	5,000
9	1,500	3,000	7,000
10	2,000	4,000	8,000
11	----	5,000	10,000

*1 unit = 0.00001 mg. protein nitrogen.

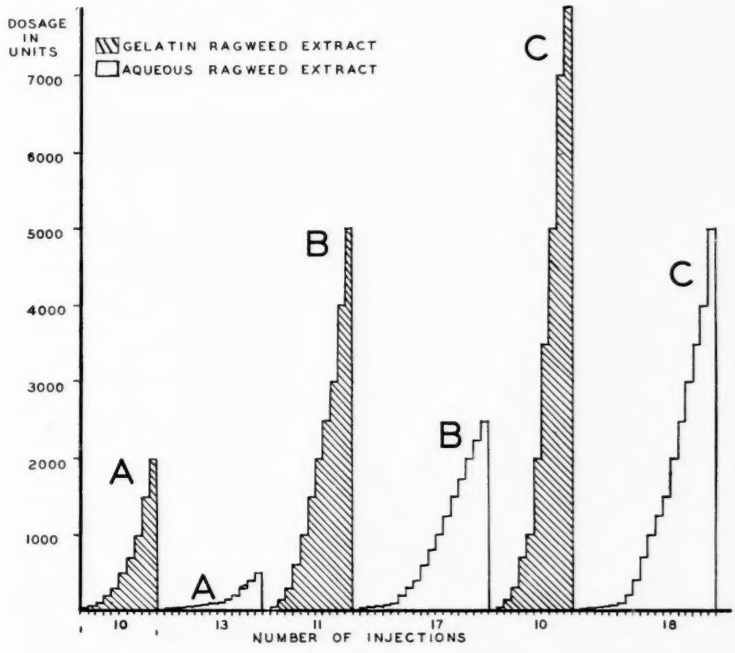

Fig. 1.

A dosage schedule for the treatment of hay fever patients by injections of pollen extract cannot be arbitrarily fixed. The schedule was often modified to suit the individual's tolerance for the gelatin-ragweed mixture. The decreases or increases in dosages and the maximum dosages were determined in accordance with local or systemic reactions of the patient. At other times alterations in dosages were made purely for purposes of study (Table IV).

TABLE IV

DOSAGE SCHEDULE (1940)[1]

(Given in Protein Nitrogen Units)

CASE NO.	INJECTION												NO. OF INJECTIONS TO REACH TOP DOSE
	1	2	3	4	5	6	7	8	9	10	11	12	
1	50	150[2]	300	600	1,000[3]	2,000	3,500	5,000[2]	7,000	8,000	10,000		11
2	50	150	300	700	1,250	2,250	3,500	5,000[2]	6,000[2]	7,000[2]	8,000[2]		11
3	50	150	300	700	1,250	2,250	3,500	5,000	7,000	8,000			10
4	50	150	300	700	1,250	2,000	3,500[2]	5,000	6,000[4]	7,000	8,000		11
5[8]	50	150	400	700[2]	1,000[2]	1,500[2]	2,500	3,500	5,000				9
6	50	150	300[2]	700[2]	1,000	2,000	3,500	5,000[2]	7,000	8,000			10
7	50	150	300	700	1,000	2,000	3,500	5,000	7,000[2]	8,000[2]			10
8	50	150	300	600	1,000	2,000	3,500	5,000[2]	6,000	7,000[2]	8,000[2]	10,000[2]	12
9[9]	200	500	1,000	2,000	3,000[2]	5,000	7,000[3]	9,000[2]	10,000[4]				9
10	50	150	300	600	1,000	1,500	2,000	3,000[2]	4,000	5,000			10
11	50	150	300	600	1,000	1,500	2,000	3,000	4,000[2]	5,000	6,000		11
12	50	150	300	600	1,000	1,500	2,000	2,500	3,500	5,000	6,000[2]		11
13	50	150	300	600	1,000	1,500	2,000	2,500	3,500	4,000	5,000		11
14	50	150[2]	300	600	1,000	1,500	2,000	2,500	3,500	5,000			10
15	50	150	300	600	1,000	2,000	3,500[2]	4,000	5,000				9
16	50	150	300	600	1,000	1,500	2,000	2,500	3,000	4,000	5,000	6,000	12
17	50	150	300	600	1,000	1,500	2,000	2,500	3,000	3,500	4,000[2]	5,000[2]	12
18	50	150[2]	300	600	1,000	1,500	2,000	2,500	3,500	5,000[2]	6,000[2]		11

19	50	150	300	600	1,000	1,500	2,000[2]	2,500[2]					8
20	50	150	300	600	1,000	1,500	2,000	3,000	4,000	5,000			10
21	50	150	400	800	1,250	1,750	2,250[2]	2,750	3,250	3,500[2]			10
22	50	150	300	600	1,000	1,500	2,000	2,500	3,000[2]	3,500[2]			10
23	25	100[2]	300[2]	600[2]	1,000	2,000[2]	3,000[2]	4,000	5,000				9
24	25	75	150	300	600	1,000	1,500	2,000	2,500	3,000	3,500		11
25	50	150	300	600	1,000	1,500	2,000[2]	2,500					8
26	25	75	150	300	500	700	1,000	1,500	2,000[2]	3,000[2]			10
27	50	150	300	600	1,000	1,500	2,000						7
28	25	75	150	300	600	1,000	1,500	2,000	2,500	3,000	3,500[2]		11
29	50	100	200	400	700	1,000	1,500	2,000	2,500				9
30	50	150	300	600[6]	400	500	600	800[2]	1,000[3]				9
31	50	150	300	500	700	1,000	1,500[6]	1,750[6]	1,500				9
32	25	75[2]	150	300	600	1,000	1,500	2,000[2, 7]	1,750[4, 6]	1,500[2]			10
33	50	100	300[2]	600	1,000	1,500	2,000[6]	1,500					8
34	25	75	150	300	600	1,000	1,500						7
35	50	100[2]	300	600	1,000	1,500	2,000	2,500[7]	2,000[6]	1,500[2]			10
36	25	50	100	300	500	600	700	800	1,000				9
37	50[6]	25	75	125	200	400	600[2]	1,000	1,500	2,000			10
38	10[5, 6]	20[5, 6]	30[5, 6]	50[5, 6]	60[5]	80[5]	100[5]	200[5]	300[5, 7]	100[5, 6]	100[5]	200[5]	
	300[5]	400[5, 7]	400[5, 7]	400	800	1,250	1,500						
39	10[5, 6]	20[5, 6]	15[5, 6]	25	75	150	300	400	600	800	1,000	1,500	
40	10[5, 6]	10[5, 6]	25	50	100	300	400	600	700	1,000			

Class C, cases 1 to 9 inclusive; Class B, cases 10 to 24 inclusive; Class A, cases 25 to 40 inclusive.

[1]Injections were given at weekly intervals, unless otherwise noted; [2]given after interval of two weeks; [3]given after interval of three weeks; [4]given after interval of four weeks; [5]aqueous extract used; [6]mild reaction occurred; [7]severe reaction occurred; [8]started treatment on July 19; [9]reached 10,000 with gelatin extract in September, 1939, and returned April, 1940.

Nine Class C patients attained a maximum dosage of 5,000 units with an average of eight injections of the gelatin extract, whereas it usually required eighteen injections of the aqueous extract to reach this maximum providing interruption of treatment did not demand a repetition of dosage. An average maximum dosage of 7,500 units was reached with an average of ten injections of the gelatin extract.

Similarly fifteen Class B patients reached 2,500 units with an average of eight injections of the gelatin extract as compared to seventeen injections required with the aqueous extract when there was no interruption of treatment. Moreover, an average of 4,800 units was reached with an average of ten injections with the gelatin extract.

The most sensitive group of patients, Class A, required an average of six injections to reach 500 units as compared to thirteen injections with the aqueous extract. An average of 1,800 units was reached with an average of ten injections. Within this group there were included several patients who exhibited higher degrees of sensitivity than the usual A patient. In Fig. 1 a comparison is made of the average dosage schedule when the gelatin extracts are used and the schedule followed with the aqueous extracts.

RESULTS OF TREATMENT

The results of treatment of the ninety-five patients (fifty-five in 1939 and forty in 1940) treated with the gelatin-ragweed pollen extract were compared with the results of ninety-five other patients treated with the regular aqueous ragweed extract during the same period. Each patient was supplied with a card upon which spaces were provided for each day of the hay fever season. He was requested to note each day the degree of hay fever present and was instructed in the proper estimation of his daily symptoms as slight, moderate, or severe.[9] An estimation of results obtained by a study of these reports shows (Table V) that, of the ninety-five patients treated with the gelatin-ragweed extract, seventy-eight, or 82.1 per cent, experienced a satisfactory degree of relief (of these seventy-eight, twenty-two had slight or no hay fever symptoms), while in seventeen, or 17.9 per cent, the symptoms, though greatly diminished, were still severe. No effort was made in these patients to treat concomitant sensitizations to other allergens which had

TABLE V

RESULTS OF PRESEASONAL TREATMENT

TREATMENT	NO. OF PATIENTS	NO OR SLIGHT HAY FEVER		NO SEVERE HAY FEVER		5 OR LESS SEVERE DAYS		SEVERE HAY FEVER		TOTAL EFFECTIVE RELIEF	
		NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
Gelatin-ragweed extract	95	22	23.1	18	19.0	38	40.0	17	17.9	78	82.1
Aqueous ragweed extract	95	9	9.5	19	20.0	41	43.1	26	27.4	69	72.6

been detected during the course of treatment. In the other group of ninety-five patients treated with the regular aqueous ragweed extracts and also with other specific excitants, 72.6 per cent were benefited while 27.4 per cent had severe hay fever. Thus the patients who were treated

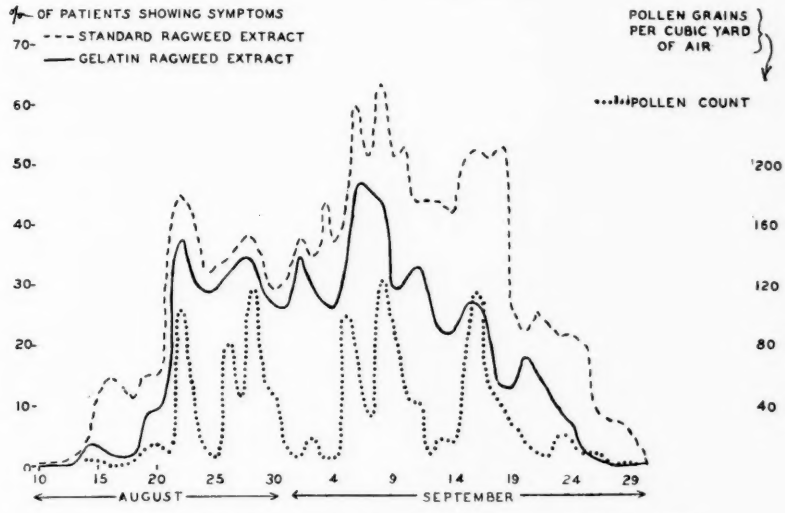

Fig. 2.—Relation of symptoms to pollen production—1939.

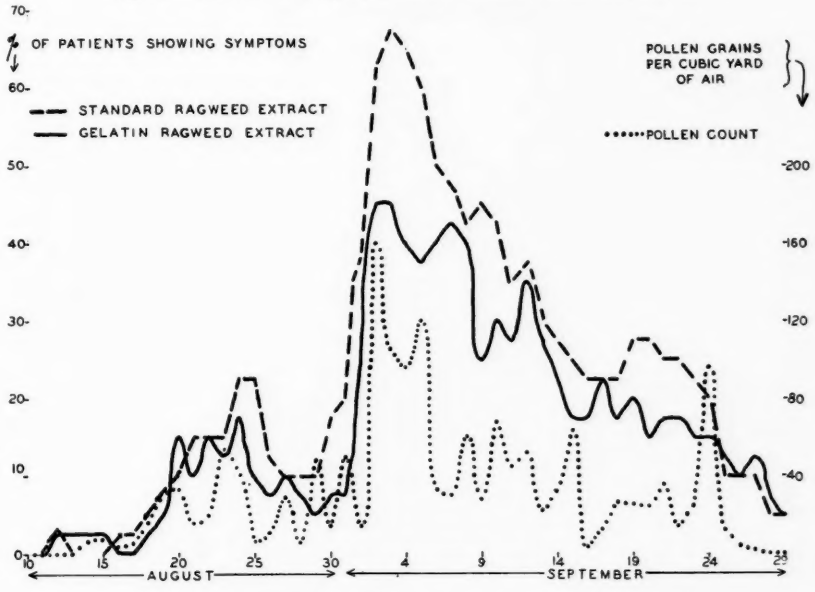

Fig. 3.—Relation of symptoms to pollen production—1940.

with the gelatin-ragweed mixture developed a greater protection than the other group. This is illustrated (Figs. 2 and 3) by a comparison of the symptom curves of both groups studied in relation to the pollen production.*

*Pollen concentration in the metropolitan area of New York City as reported by Abbott Laboratories.

Of the twenty-two patients treated by the perennial method with injections of maximum or near maximum doses of the gelatin-ragweed pollen extract, twenty-one, or 95.5 per cent, experienced effective relief.

LOCAL AND CONSTITUTIONAL REACTIONS

The local reactions from injections with the gelatin-ragweed extract attained their maximum size in about one-half to three hours after injection. The sites of injection were at first moved from one arm to the other, but this plan was abandoned shortly afterwards since there seemed to be no advantage in continuing such a procedure. Untoward reactions were infrequent and, when present, were delayed and mild. Of the ninety-five patients treated with the gelatin-ragweed extract, three (one in 1939 and two in 1940) had constitutional reactions necessitating the use of epinephrine. Nine other patients (five in 1939 and four in 1940) showed mild reactions which were not severe enough to need epinephrine. All of these reactions occurred because dosages were greatly increased in an effort to determine a proper schedule.

The following are examples of typical reactions:

CASE REPORTS

CASE 37.—F. G. had been treated for hay fever symptoms with the aqueous extracts since 1936 and had experienced frequent severe reactions. In 1938 she reached 400 units with difficulty, severe local reactions occurring. In 1939 she reached only 150 units. In 1940 treatment was begun with 50 units of the gelatin-ragweed pollen extract. A marked local reaction appeared two hours later and persisted for two days. There were no systemic reactions. One week later the patient received 25 units with no ill effects. A top dosage of 2000 units was reached with nine injections.

CASE 31.—B. M. had been receiving treatment with the aqueous extracts since 1932. Although classified as a B case, she demonstrated a Class A sensitivity clinically. She experienced frequent constitutional reactions, and dosages were often repeated or reduced. The maximum dosage attained was 1,000 units and was followed by a severe constitutional reaction. This year she received injections of the gelatin-ragweed pollen extract at weekly intervals, as follows: 50, 150, 300, 500, 700, 1,000, and 1,500 units. A marked local reaction appeared one hour later at the site of this last injection and lasted for two days. One week later, 1,750 units were injected for purposes of study. A marked local reaction again resulted at the site of injection. The patient complained of slight itching of her palms, and several hives appeared over both arms. All these symptoms subsided within two hours without resort to any medications. A week later the dosage was reduced to 1,500 units and maintained at that level.

CASE 35.—S. M. had been treated since 1931 with the aqueous extracts. Although classified as B by intracutaneous tests, she was treated according to the schedule for A cases. She experienced frequent severe reactions necessitating epinephrine when dosages greater than 700 units were given. This year she received injections of the gelatin extracts as follows: 50, 100, 300, 600, 1,000, 1,500, 2,000 and 2,500 units. One hour after the last injection hives appeared over both arms, and she experienced typical hay fever symptoms. A dose of 0.2 c.c. of

epinephrine was given, and the symptoms subsided within one-half hour. One week later the injection was reduced to 2,000 units, and a marked local reaction occurred, lasting for three days. Two weeks later she was given 1,500 units, and a moderate local reaction appeared on the site of injection. This last dosage was considered the top dose.

In several instances in the course of treatment, injections of the aqueous ragweed pollen extract were substituted for the gelatin-ragweed pollen extract. The differences in effect of the two extracts upon the patient are shown in the following instances. In patient A. B., a marked local reaction occurred when 30 units of the aqueous extract were administered, whereas no such reaction occurred when the same dose of the gelatin extract was injected the previous week. When a dose of 2,000 units of the aqueous extract was substituted in patient J. J. a week after the same amount of gelatin extract had been injected without ill effect, a severe local reaction occurred not only in the arm injected but also in the opposite arm. Another patient, A. B., was receiving 3,000 units of the gelatin extract once a month. Upon injection of 3,000 units of the aqueous extract, a severe local and a constitutional reaction followed immediately, the hay fever symptoms, generalized urticaria and asthma, persisting for two days. Subsequently 3,000 units of the gelatin extract were injected with safety and repeated each month.

The gradual diminution in activity of pollen extracts was recognized, so that upon changing to a new extract a reduction in dosage was required. Since the gelatin extracts were more slowly absorbed, it was possible to change directly from the old to the new without lessening the dosage and with no untoward reactions.

DISCUSSION

By the addition of gelatin to the standard aqueous pollen extracts, the number of injections required for hay fever treatment is often reduced one-half, and the probability of constitutional reactions is diminished. Furthermore, the maximum dosage is often twice the amount attained with the aqueous pollen extracts, and the injection interval may be lengthened.

The patients selected for treatment with the gelatin extracts consisted largely of those who were unable to undergo satisfactory treatment with the aqueous extracts because of their marked degree of sensitiveness. Treatment with the gelatin-pollen extracts seems to have given more effective relief than the standard aqueous pollen extracts. No uniform schedule for administration of the gelatin-ragweed pollen extracts was adopted during this study. It was preferred to determine the degree of dosage increase possible in each patient. Furthermore, injections at intervals of from one to four weeks were found feasible. With such an experimental procedure local and systemic reactions were occasionally

produced. An average dosage schedule for prophylactic hay fever treatment was tabulated. This schedule was modified according to the patient's tolerance for the gelatin-pollen extract. Decreases or increases in dosages and the maximum dosages were determined by the extent of the local reaction or by any undesirable systemic reaction. No local irritation or induration at the sites of the injections occurred.

The disadvantages incident to the use of the gelatin extract are the inconvenience of liquefying it before administration and the possibility of a delayed constitutional effect occurring many hours after the injection, when the patient is no longer under the observation of the physician. While these reactions have so far been found to be mild and infrequent, they might be severe enough to require epinephrine for relief. The use of this extract will not obviate a reaction resulting from an accidental intravenous injection. The gelatin mixture which is used as a coreagent with a standardized ragweed extract must be carefully made following the directions as outlined by Spain, Strauss, and Fuchs.[6] In autoclaving, for example, the time and pressure advised (twenty minutes at 30 pounds pressure) should not be exceeded, otherwise the gelatin will lose some of its properties. It is also important that all ingredients in the mixture be included and that strict precautions for sterility be observed. Serologic studies of the immunologic mechanism[10, 11] on the patients treated with the gelatin-pollen extract are now being made to determine whether the injection of maximum dosages of the slowly absorbed extract will afford a more continuous and sustained protection from the pollen.

SUMMARY

1. Gelatin, a slow absorption medium, was mixed with ragweed pollen extracts in an attempt to overcome difficulties encountered with the standard aqueous extracts.

2. A delay in absorption of the active ragweed substance of the gelatin-ragweed extract as compared to the aqueous ragweed extract was noted following subcutaneous injections at passive transfer sites in a series of normal nonsensitive adults.

3. A series of ninety-five adult patients suffering from ragweed hay fever symptoms were treated preseasonally with the gelatin-ragweed extracts. Another group of ninety-five adult patients were treated with the standard aqueous extracts during the same period. Twenty-two patients of the first group continued their treatment on a monthly basis during 1940 after having completed a course of preseasonal injections.

4. A greater tolerance for the ragweed-gelatin extract was noted; higher maximum dosages (often twice the amount attained with the aqueous extracts) were given with safety and with half the number of injections.

5. Constitutional reactions were milder and occurred infrequently.

6. More effective clinical relief was obtained following treatment with the gelatin-ragweed extracts.

7. An average dosage schedule for preseasonal treatment with gelatin-pollen extracts was determined. It was modified to suit the individual's tolerance.

8. The gelatin extract must be prepared according to the technique outlined if standard and satisfactory results are to be obtained.

9. At present the chief value of the gelatin extracts is in the treatment of hay fever patients so sensitive as to accept poorly the usual aqueous pollen therapy.

10. Further studies are in progress to determine whether the use of the more slowly absorbed gelatin extracts will result in a more sustained and lasting immunity.

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THE TREATMENT OF HAY FEVER BY INJECTIONS OF SUSPENDED POLLEN TANNATE

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THE treatment of pollen sensitivity by the subcutaneous injection of increasing doses of aqueous pollen extracts has proved to be the only generally effective method of treatment. The fundamental principles governing the preparation and administration of pollen antigens were first established by Noon[1] in 1911 and consist of two factors: (1) preparation by the extraction of the specific antigen by water and (2) administration by the injection of gradually increasing amounts of the specific antigen. Attempts to improve the treatment by modifying the factor of administration have been numerous and include such variations as intradermal injection, scarification of the skin, the use of electrical currents to force the antigen through the skin, application of the antigen to the nasal mucous membrane, and ingestion of the antigen. The subcutaneous injection remains the generally accepted method of administration. Likewise, the preparation of antigens still depends on the use of water for extraction although variations such as the addition of glycerin, dextrose, alcohol, buffering salts, etc., have been recommended. Significant improvement in the treatment of pollen sensitivity would seem to require a greater change in the method of preparation of antigens. Unsuccessful attempts[2] have been made by modifying pollen extract by formalin, heat, acetylation, ultraviolet radiation, and adsorption on precipitated alum.

In recent years there have been many examples of the increased effectiveness of injected substances when their absorption has been slowed. It seems possible that the treatment of pollen sensitivity might be improved if the active material in pollen extracts could be so modified as to be absorbed slowly. One method of accomplishing this is the adsorption of pollen extract on precipitated alum.[3] This method has shown no clinical advantage over the usual aqueous solutions probably because the active material is present on the surface of the particles and thus comes into contact with antibodies immediately after injection. The injection of pollen extracts emulsified in lanolin and oil so that each droplet of aqueous extract is covered by a layer of lanolin and oil offers a different method of slowing absorption.[4]

The possibility was considered that active material precipitated from pollen extract might have advantages similar to those of in-

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sulin precipitated with protamine.[5] Preliminary experiments were made with formalin-treated precipitates obtained by adding alcohol or acetone to aqueous pollen extracts. Later it was learned that tannic acid had been used to precipitate scarlet fever toxin without decreasing its antigenicity.[6a, b] Tannic acid precipitates of insulin[7] and more recently of pitressin[8] have also been used successfully. Accordingly, it was decided to try tannic acid as the precipitant of pollen extract.

The purpose of this paper is to present the method of precipitation by tannic acid of the active material in pollen extracts and the results obtained in the treatment of hay fever with the precipitates.

METHODS

Preparation of Materials.—During 1939 preparations were made by adding an excess of a fresh tannic acid solution to a dilution of pollen extract. Usually a final concentration of 0.5 per cent to 1.0 per cent of tannic acid was sufficient to produce complete precipitation. The resulting precipitate was centrifuged, the supernatant decanted, and the precipitate suspended in the desired volume of Coca's buffered solution. All procedures were carried out under sterile precautions. A typical protocol is given for illustration.

Five grams of mixed giant and dwarf ragweed pollen, after being defatted with ether, were suspended in 100 c.c. of an equal mixture of Coca's solution and glycerin to make a 1:20 extract. The extract was Seitz filtered, and 80 c.c. were treated with 8 c.c. of a 10 per cent tannic acid solution in distilled water. Part of the precipitate was centrifuged two days later and the remainder on the third day. The entire precipitate was then resuspended in 40 c.c. of Coca's solution to make a preparation designated as ragweed pollen tannate 1:10.

A 1:10 dilution with Coca's solution of the ragweed tannate 1:10 was called ragweed tannate 1:100. Suspensions of pollen tannate 1:10, 1:100, and 1:1,000 were used in treatment. Precipitates of the following pollens were prepared: (1) birch, (2) oak, (3) timothy and mixed grasses in the proportion of 50 per cent timothy and 50 per cent mixed grasses (sweet vernal, red top, June, Bermuda, and orchard), (4) ragweed, giant and dwarf mixed in equal quantities.

During 1940 the same procedure was followed as in 1939, except that pollen extracts were made without glycerin, and the precipitate formed after the addition of tannic acid was dried by acetone, or alcohol followed by ether. It was then ground in a mortar to a powder and stored in a desiccator. When desired, the powder was weighed on a chemical balance and suspended in Coca's solution. Suspensions of 2.0 mg., 0.2 mg., and 0.02 mg. per cubic centimeter were used. It was calculated that 2.0 mg. of the dry pollen tannate was approximately equivalent to 1 c.c. of the 1:10 suspension used in 1939.

The supernatant remaining after the tannic acid precipitation did not produce scratch skin reactions. The tannate suspensions gave positive skin reactions which were smaller than the reactions produced by the original extract. The supernatants from such tannate suspensions when further treated with tannic acid showed precipitation again and the skin reactivity became decreased or negative.

The ability of the tannate suspension to produce positive skin reactions did not change on standing. A suspension 5 months old produced the same skin reaction as a suspension 1 day old. Total nitrogen determinations showed comparable results on suspensions standing one hour, one day, and five months. Such analyses were made of the entire suspension and of the precipitate remaining after the supernatant was removed (Table I).

TABLE I

MILLIGRAMS OF TOTAL NITROGEN IN 1940 RAGWEED TANNATE SUSPENSIONS

0.5 C.C. OF 2.0 MG. PER C.C. SUSPENSION	1 HOUR	1 DAY	5 MONTHS
Whole suspension	0.0914	0.1036	0.1007
Precipitate only	0.0508	0.0625	0.0540

Selection of Patients for Treatment.—Two criteria were considered in the selection of patients for treatment: (1) severity of symptoms and (2) degree of sensitivity as shown by the scratch skin test. Only patients with moderate to severe hay fever, with or without associated asthma, were accepted. All patients were skin tested by the scratch method with 1:50, 1:500, and 1:5,000 dilutions of regular pollen extract in Coca's solution before treatment, and only those with moderate to large skin reactions were treated. Seventy-seven ragweed-sensitive patients were included in this series.

In 1939 fifty-five patients were treated for ragweed sensitivity, twenty-nine in the clinic and twenty-six privately. There were twenty-four males and thirty-one females, ranging in age from 16 to 51 years. The duration of symptoms ranged from one year to twenty-five years. Thirty patients had had previous treatment for one or more years with ragweed emulsion in oil; fourteen had no previous treatment; eleven had had treatment one or more years previously with solutions of ragweed extract. Seven patients had injections of ragweed emulsified in oil just before ragweed tannate was started, which probably had some effect on the subsequent desensitization. Since the last injection of the emulsion was given four to five months before the season, the effect on the clinical results was at most very slight.

In 1940 forty-eight patients were treated for ragweed sensitivity, twenty-four in the clinic and twenty-four privately. There were twenty-seven males and twenty-one females, ranging in age from 9 years to 61 years. The duration of symptoms ranged from one year to thirty or more years. Twenty-four patients had a full course of treatment in 1939 with ragweed tannate, and two patients had a partial course of

treatment; seven patients had had previous treatment with regular extracts and fifteen patients had had no previous treatment.

Some of the patients treated with ragweed tannate had multiple pollen sensitivity and were also treated with the specific pollen tannates involved. Other patients were treated only with grass pollen tannate. Since the number of patients treated with tannate precipitates other than ragweed was small and the seasons were shorter and milder than the ragweed season, only the results of the ragweed treatment are presented.

Method of Treatment.—The injections were given subcutaneously in the upper arm once or twice a week. The doses were increased according to the degree of reaction to the last injection so that no regular schedule of dosage was observed. When the maximum dose was reached, it was repeated at intervals of one to four weeks until the middle of August when the season started. In many cases injections were also given during the season to the middle of September, usually with a reduction in dosage.

In 1939 the patients receiving treatment for the first time and the patients who had had previous treatment with regular extracts were started with the 1:1,000 suspension, usually 0.1 c.c. The patients who had had treatment in 1939 with ragweed emulsion in oil were started with larger doses, ranging from 0.2 c.c. of 1:1,000 to 0.3 c.c. of 1:100. The maximum dose was generally 0.5 c.c. of the 1:10 suspension.

In 1940 the patients who had had treatment the previous year with ragweed tannate were started generally with 0.1 c.c. of the suspension containing 0.2 mg. per cubic centimeter. All other patients were started with the 0.02 mg. per cubic centimeter suspension, 0.1 c.c. being the usual amount given. The maximum dose was 0.5 c.c. of the suspension containing 2.0 mg. per cubic centimeter, i.e., 1.0 mg. of dry pollen tannate.

RESULTS

In order to obtain a comprehensive view of the effect of treatment with the pollen tannate suspensions, the findings have been analyzed according to the following criteria: (1) number of injections, (2) evidence of hyposensitization, (3) clinical results, and (4) duration of hyposensitization.

Number of Injections.—Of the fifty-five patients treated with ragweed tannate in 1939, seven had enough treatment with ragweed emulsion before the tannate was started to affect the dosage with tannate; therefore these cases were excluded from an analysis of the number of injections required to effect hyposensitization. The average values for the remaining forty-eight patients are given in Table II. The type of treatment in previous years had no apparent influence on the treatment with tannate extracts.

TABLE II

RAGWEED TANNATE TREATMENT IN 1939 AND 1940

YEAR	PREVIOUS TREATMENT	NO. OF PATIENTS	MAXIMUM DOSE*	INJECTIONS TO MAXIMUM DOSE	TOTAL NO. OF INJECTIONS
1939	None (1)	14	0.46	13.7	16.1
	Regular extract (2)	7	0.43	16.4	17.4
	Combined 1 and 2	21	0.45	14.6	16.5
	Emulsified extract	27	0.47	13.3	15.1
	All cases	48	0.46	13.6	15.7
1940	None (1)	12	0.44	15.4	18.0
	Regular extract (2)	7	0.38	13.7	17.1
	Combined 1 and 2	19	0.42	14.2	17.6
	Tannate, 1939	23	0.46	8.3	11.6
	All cases	42	0.44	10.0	14.9

*In 1939, maximum dose in c.c. of 1:10 suspension; in 1940, maximum dose in c.c. of 2.0 mg. per c.c. suspension.

Of the forty-eight patients treated with ragweed tannate in 1940, six had treatment with a modified formalin tannate preparation before the regular tannate was given, and these cases are excluded. The average values for the remaining forty-two cases are given in Table II.

Evidence of Hyposensitization.—Two methods were used to indicate hyposensitization after the course of treatment with the tannate: (1) diminution in the size of the skin tests and (2) the reaction to large doses of regular ragweed extract.

Before treatment all the patients were scratch skin tested on the forearm with regular extract 1:50, 1:500, 1:5,000, and in most instances the tests were repeated. In practically all cases, the diameters of the wheals and surrounding zones of erythema were measured. In 1939 thirty-four patients had tests done before and after treatment; twenty-seven of these had marked reduction of the skin reactions, and seven had slight to moderate reduction. In 1940 twenty-nine patients were tested before and after treatment; nineteen had marked reduction in the skin reactions, six had slight to moderate reduction, and four had none.

In 1939, thirty-one of the fifty-five cases were given subcutaneously from 0.1 c.c. to 0.35 c.c. of regular ragweed extract 1:50 to test their hyposensitization, and in practically all instances only slight to moderate local reactions occurred. In 1940 no patients were tested in this manner because it was considered that the results in 1939 were proof enough of the hyposensitizing activity of the ragweed tannate suspension.

Clinical Results.—The classification of clinical results is difficult because of the subjective evidence upon which it is based. However, four classifications have been made, the results in each case being estimated as conservatively as possible. In general, the excellent group includes patients who had no symptoms to mild ones for short periods on a few scattered days during the season. The good group includes patients with scattered mild symptoms but in addition an occasional day of

more severe ones. The fair group includes patients with periods of more marked discomfort and up to seven days of moderate hay fever, but with decided improvement as compared with previous years. The poor group includes one patient described below.

The clinical results are known in fifty-three of the fifty-five patients treated in 1939. At the end of the course of treatment with tannate extracts, ten of the patients also had four to nine regular extract injections, starting with 0.1 c.c. of 1:50 to 0.125 c.c. of 1:20 and ending with 0.3 c.c. of 1:50 to 0.4 c.c. of 1:20. Six of these ten had excellent results; three had good results; and one had fair results. Of the remaining forty-three patients, twenty-two had excellent results, seventeen had good results, and four had fair results (Table III).

TABLE III

CLINICAL RESULTS IN PATIENTS TREATED IN 1939 AND 1940 WITH RAGWEED TANNATE

YEAR	NO. OF PATIENTS	EXCELLENT		GOOD		SATISFACTORY (EXCELLENT AND GOOD)		FAIR		POOR	
		NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
1939	43	22	51.16	17	39.53	39	90.69	4	9.30	0	0
1940	39	22	56.41	13	33.33	35	89.74	3	7.64	1	2.05

In 1940 the clinical results are known in forty-one cases. Two patients completed their treatment with tannate early and, because they had to leave, were given regular ragweed extract 1:50 to take with them to maintain hyposensitization. Both had excellent results. Of the remaining thirty-nine patients (Table III), twenty-two had excellent results, thirteen had good, three had fair, and one had poor results.

Of the patients with fair results in 1939, four had good hyposensitization, as indicated by the tolerance to large doses of regular ragweed after treatment, and diminution in skin tests. The fifth patient was not tested, but was well hyposensitized, as shown by a small reaction to 0.1 c.c. of regular ragweed extract 1:100, when he reported for treatment in 1940. Three of the patients had had very severe symptoms for a great many years, and, although they had marked improvement as compared with previous years and were satisfied with the degree of improvement, they had enough symptoms to exclude them from the group of good clinical results. The remaining two patients probably had other sensitivities since they had excellent objective hyposensitization. In one patient hyposensitization persisted as shown by small skin reactions the following year at which time she tolerated 0.1 c.c. of 1:100 regular ragweed extract. She had poor results in 1940 with a final dose of 1.0 c.c. of 1:100 regular ragweed.

Of the three fair cases in 1940, two had fair results in 1939 and one had insufficient treatment. The poor case in 1940 cannot be explained.

He had excellent results in 1938 with emulsion treatment, good results in 1939 with tannate, and in 1940 no evidence of hyposensitization with tannate treatment.

Duration of Hyposensitization.—The duration of hyposensitization produced by ragweed tannate treatment in 1939 was determined by (1) the tolerance to large doses of regular ragweed extract when the patients returned for treatment the following year, six months or more after the last injection, (2) the number of injections required in 1940 to reach the maximum dose in patients who had ragweed tannate in 1939 as compared with patients who did not have previous treatment with ragweed tannate, and (3) the degree of hay fever in patients who had no treatment in 1940.

Nineteen patients treated with tannate in 1939 were injected with regular ragweed extract 1:100 when they returned for treatment in 1940; eighteen received 0.10 c.c., and one received 0.05 c.c. The local reactions were negative to moderate in all cases, and one had a very slight general reaction which occurred after leaving the clinic and required no treatment. Three patients received 0.1 c.c. of 1:300 regular extract, and one patient 0.1 c.c. of 1:500, with negative to moderate local reactions. Only enough patients were tested in this manner to indicate that a fairly marked degree of hyposensitization persisted after treatment with ragweed tannate. Since not all cases were so tested and the maximum degree of tolerance was not determined, a complete statistical analysis is not possible.

Another indication of persisting hyposensitization is obtained by dividing the 1940 cases into two groups: (1) patients treated with ragweed tannate in 1939 and (2) patients not treated previously with ragweed tannate (Table II). The patients treated with tannate extracts in 1939 required approximately one-half as many injections in 1940 to reach the average maximum dose as the group with no previous tannate treatment.

Fifteen patients treated with tannate extracts in 1939 are known to have had no treatment of any kind in 1940. Four of these patients had four to five injections of regular ragweed extract in addition to their tannate treatment; three had practically no symptoms in 1940, and one was very uncomfortable. In the remaining eleven cases, the symptoms were negligible to slight in nine, moderate in one, and severe in another.

DISCUSSION

The first question to be answered was whether tannic acid precipitated the active material from pollen extracts. Since the supernatant fluid remaining after precipitation no longer gave positive reactions in sensitive patients, it was considered that the active material came down with the precipitate. That the active material was present in the precipitate was proved by the results of treatment of sensitive patients with the precipitate, namely, (1) the production of local and general reactions,

(2) diminution in the size of skin tests, (3) tolerance to large doses of whole pollen extract, and (4) satisfactory clinical improvement. The nature of this active substance is a matter of importance. Whether the active principle in pollen extract is a protein has always been a subject of speculation. It is possible that the active principle was brought down with the tannic precipitate by adsorption. It is more likely, however, that the active principle is a protein precipitable by tannic acid. The hyposensitization to whole pollen extract produced by the tannic acid precipitable portion of the extract affords evidence that pollen sensitivity is a protein sensitivity.

A statistical comparison of the effectiveness of treatment with pollen tannate and the various extracts now in general use would require a large series of cases treated the same year, or over a number of years. In general, patients previously treated with regular pollen extract claimed better results with the tannate preparations. That treatment with the pollen tannate is effective is shown by the high percentage of satisfactory results (90 per cent) obtained with a relatively small number (fifteen) of injections. Of even greater significance is the evidence of persistence of hyposensitization as indicated by the facts that (1) many patients were able to tolerate large doses of regular ragweed extract (0.1 c.c. of 1:100) six months or more after the last treatment with tannate, (2) during the second year of treatment the average number of injections necessary to reach the average maximum dose (8.3 injections, 0.92 mg.) was approximately one-half the average number required for those being treated with tannate for the first time (14.2 injections, 0.84 mg.), and (3) the degree of relief from hay fever without treatment the season following a course of pollen tannate injections was satisfactory (twelve of fifteen patients had negligible to slight symptoms). The impression gained is that pollen tannate treatment produces a greater degree of hyposensitization with fewer injections than the treatment with regular extracts and that it is more persistent.

The use of dried pollen tannate offers an advantage for purposes of standardization. There are three methods of standardization in use at present, namely, (1) the weight/volume relationship, as indicated by the Noon unit[1] or the dilution system, (2) the estimation of total nitrogen in the extract,[9] and (3) standardization by determination of protein nitrogen.[10] Three different methods of expressing quantities of pollen present an obvious source of confusion. The use of weighed active material would provide a more direct and simpler method of expressing dosage.

Another advantage of dry pollen tannate concerns the element of stability. Pollen extract in solution tends to lose antigenic activity. Many modifications of extracting fluids have been recommended to prevent this, with varying degrees of success. It is probable that pollen tannate under proper conditions of dryness would keep indefinitely. It is

also possible that the factors responsible for the deterioration of the antigenic activity (enzymes?) may be eliminated in the process of precipitation or may be less active in the changed physical conditions of the antigen, so that, when the pollen tannate is suspended, no loss of activity may occur.

When tannic acid is added to pollen extract, the supernatant loses its ability to produce scratch skin reactions. It was expected that the resuspended precipitate would also fail to give scratch skin reactions since all the antigen had been precipitated. However, the suspension did produce skin reactions. The supernatant of such a suspension likewise produced skin reactions and showed a precipitate when treated with protein precipitants. The probable explanation for this is the dissociation of some of the precipitated protein tannate into free protein ions and free tannic ions as follows:

$$\text{Protein tannate} \rightarrow \text{protein ions}^{+} \text{ and tannic ions}^{-}$$

The free protein ions in solution could then react as in the original extract. Because of this dissociation, local and general reactions often appeared as quickly after injection with protein tannate as with regular aqueous extracts.

The chief advantage of using precipitated material depends upon gradual dissociation and absorption occurring after injection, thus preventing severe, immediate, local and general reactions occurring from contact between the tissues and excessive amounts of free active antigen. It is important to reduce to a minimum the degree of such dissociation occurring before injection. Preliminary experiments suggest that, before tannic acid is added, the addition to the pollen extract of zinc chloride to a concentration of 0.1 per cent will help to do this. The supernatant of suspensions of zinc pollen tannate gave negative scratch skin reactions and showed only a minimal or no precipitate with protein precipitants. Such suspensions injected intradermally showed positive reactions which were slow in development and increased gradually. Zinc salts added to gonadotropic extracts[11] have increased their effectiveness by prolonging their absorption, and zinc has been added to the protamine insulin now being used. Suspensions of the dry precipitate in oil would also probably prevent dissociation and might help to retard absorption.

The necessity of powdering the dried protein tannate in a mortar may be eliminated by the use of the lyophile apparatus to dry the centrifuged precipitate in the frozen state. Lyophilized precipitate yields a finer, lighter powder than that produced by manual trituration.

Stock suspensions which probably would be stable may be made more simply by adding an equal volume of sterile glycerin to the original centrifuged sediment. Such a suspension could be standardized by the total nitrogen content, and dilutions made as desired with buffered saline solution.

SUMMARY

1. The active material in pollen extract may be precipitated as a tannate by tannic acid in excess.

2. Pollen tannate resuspended in buffered saline dissociates partially but not progressively.

3. Seventy-seven ragweed-sensitive patients were treated in 1939 and 1940 with injections of ragweed tannate suspensions. Ninety per cent showed satisfactory clinical results with an average total of fifteen injections. Only one patient failed to show evidence of hyposensitization to whole pollen extract. Many patients treated in 1939 showed evidence of hyposensitization six months or more after the last injection. In twelve of fifteen patients treated in 1939 alone, the hyposensitization persisted through the ragweed season.

4. The gradual dissociation and absorption of the active material in pollen tannate after injection apparently produces a greater degree of hyposensitization with fewer injections than regular pollen extract.

5. The effective hyposensitization of a large series of pollen-sensitive patients by the tannic acid precipitate from pollen extract affords strong evidence that pollen sensitivity is a protein sensitivity.

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A STUDY OF CLINICAL SENSITIVITY TO AIR-BORNE MOLDS

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IN A previous study the incidence of positive skin tests to air-borne molds in a group of 526 patients was reported.[1] The present communication is concerned with attempts to discover and correlate clinical manifestations of sensitivity to molds with skin sensitivity in patients selected from the above group.

All of the patients in the original group had asthma or hay fever, and with two exceptions all reacted to other allergens. Eighty-five per cent of them developed positive skin reactions to one or more of fifty-two mold extracts. These patients were tested and treated over a period of three years, during which concurrent studies of the atmospheric molds were made. They were tested and treated with molds most commonly found in the air, several strains of each most frequently encountered being employed. Thus, patients were tested with five strains of *Alternaria,* six of *Hormodendrum,* four of *Aspergillus niger,* two of *Aspergillus fumigatus,* and three other strains of *Aspergilli.*

It was not assumed that 85 per cent of the 526 patients were clinically sensitive to molds, nor was it assumed that patients who gave violent positive skin tests to molds were clinically sensitive. Patients exhibiting marked positive reactions to several or all strains of one species or genus, however, were viewed with more suspicion than were those reacting to only one strain of a species or genus.

Skin sensitivity to one or more molds probably means no more than skin sensitivity to one or more foods. As is well known, skin reactions to foods and other allergens are elicited in a large percentage of people tested. Thus, Rackemann and Simon[2] obtained reactions in thirty of sixty normal nonallergic persons. Nine common allergens were used. In our experience reactions to one or more mold extracts failed to occur in only 15 per cent of allergic persons. Skin reactions at best are only clues in relation to the existence of clinical sensitivity.

METHODS AND RESULTS

In this study we have employed several approaches to the problem of demonstrating clinical sensitivity to molds. First, we noted and evaluated significant facts in the patient's history which might indicate mold sensitivity. Special attention was directed to the time factor in relation to the occurrence of symptoms and the presence of molds in the air. Second, we made an effort to evaluate the significance of

positive skin tests by performing passive transfer experiments. Third, we recorded the occurrence of systemic reactions resulting from testing and treatment with mold extracts. Other instances appear in the literature.[3-5] Fourth, we performed provocative tests, to determine in which patients symptoms of asthma and hay fever could be induced when mold spores or extracts were applied directly to the mucous membranes, as has been reported previously.[4-7]

1. Indications of Mold Sensitivity Obtained From the History and Chronology of the Patient's Symptoms.—It seemed probable that careful notes on symptomatology during the season when molds were known to be abundant in the air would be helpful diagnostically. As indicated by our mold survey, and as pointed out by Feinberg,[8] Prince and Morrow,[9] and others, mold spores are more abundant in the air during dry hot weather. In Nashville they are found to be most abundant from May to October inclusive. From the end of June until August 15 there are relatively few pollen granules in the air. Many pollen-sensitive patients experience clinical improvement in July and early August. Therefore, careful notes were recorded on patients who developed continued or increased symptoms in midsummer. On certain days during the summer the mold counts determined by the slide and plate methods were unusually high. These high counts usually held for several molds, but occasionally there were "storms" of a single variety. We noted these occurrences and attempted to relate them to the development of excessive or unexplained symptoms in certain patients.

A group of forty-three patients skin-sensitive to molds experienced continuous symptoms throughout the summer. This group did not include patients who had symptoms the year around. Eleven of the forty-three patients were 12 years of age or younger. Twenty-eight of the group exhibited two-plus skin reactions to one or more molds; and thirteen of the twenty-eight, two-plus reactions to *Alternaria*. The remaining fifteen patients gave one-plus reactions to one or more molds. On July 24, 1939, one of the two days on which we observed the unusually high *Alternaria* spore count of thirty-two (slide method), ten patients who were skin sensitive to *Alternaria* complained of unusually severe symptoms. Five of these were typical ragweed patients who had not previously suffered except at the ragweed seasons. Seven additional patients who were skin-sensitive to molds but not to *Alternaria* complained of excessive trouble on July 24, and it was thought that this group of seven patients, all of whom were skin-sensitive to other molds, might have been exposed to some molds which we were not able to identify from the slides or to *Helminthosporium*, *Acrothecium*, or *Spondylocladium* which were found present in the air in smaller numbers.

Records were kept of patients with allergic disease who lived in damp, moldy old houses, of which there are an abundance in Tennessee.

Houses in which moldy wood was stored in basements also excited suspicion. Patients who lived on farms were questioned regarding the influence on symptoms of proximity to corn cribs or piles of corn cobs, or of trips to the cornfield when the corn was in the ear. Eight patients who were skin sensitive to molds gave a history of experiencing increased symptoms when exposed to excessive amounts of molds in locations where it is known that molds are abundant (Table I).

TABLE I

PATIENTS SUFFERING ALLERGIC SYMPTOMS WHEN EXPOSED TO MOLDS

PATIENT	DIAGNOSIS	SOURCE OF SPECIAL EXPOSURE TO MOLDS
R. A., 6	Asthma	Asthma from eating certain cheeses; no trouble from milk.
G. C., 74	Urticaria and hay fever	Basement full of moldy wood—repeated Penicillium colonies on plates exposed two minutes in basement. Recovered when wood was removed.
E. C., 80	Asthma	Lived in old moldy house; stopped mold treatment because of repeated systemic reactions night of treatment.
G. F., X2	Asthma	First attack of asthma at 2 years of age, when playing with pile of corn cobs. Always trouble from corn cribs or corn.
L. H., 167	Asthma	Severe asthma on two nights when going into damp house which had been shut up (Florida).
L. S., 315	Asthma and dermatitis	Moldy house. Penicillium recovered on plates.
E. W., 414	Hay fever	Symptoms in corn field when corn in ear.
C. M., 232	Asthma	Severe attack on sea coast.

Exposure of allergic patients to environments conducive to mold growth.

Finally, some of the most impressive evidence of mold sensitivity was afforded by the favorable response of patients to mold treatment. Seventeen patients experienced definite improvement after mold extracts were added to their treatment. The improvement was prompt and occurred at a time when no other change was made in treatment. Eight of these were ragweed patients whose response to therapy was greatly improved after the addition of mold extracts. Six were children under 12 years of age, two of whom experienced symptoms in the fall only.

A patient who gave a history of symptoms when in an environment conducive to mold growth was improved by mold injections. Subsequently this subject was entirely relieved when moldy wood, from which *Penicillium* was repeatedly cultured, was removed from the basement of her home. Three other patients living in mold infested invironments improved satisfactorily under hyposensitization treatment.

Altogether, sixty-seven patients gave histories strongly suggestive of mold sensitivity. This number is derived from forty-three who developed unexplained symptoms in midsummer, seventeen who developed unusual symptoms July 24, 1939, eight who suffered unusual symptoms in places abounding in molds, and seventeen who noted marked improvement when molds alone were added to their treatment.

2. Passive Transfer Experiments.—Eighteen patients were selected for passive transfer experiments. Serum from each of these was in-

jected into the skin of two or more of twelve nonallergic subjects, according to the Praussnitz-Küstner technique. Tests were then performed at the sites of injections not only with the mold extracts which had given direct skin reactions in the serum donors, but also to dust, ragweed, or both. Of the eighteen sera, thirteen produced positive indirect reactions for one or more molds. Eleven of these thirteen sera caused one or more two-plus direct skin reactions to molds, yielding forty two-plus reactions, thirty-five of which could be transferred to the skin of nonsensitive persons.

The thirteen sera produced one-plus skin reactions to molds in thirty-three instances, and only fifteen of these reactions could be transferred to the skin of nonsensitive persons. Sera from five patients failed to produce indirect skin reactions, and in this group there had occurred four two-plus direct skin reactions and twelve one-plus direct skin reactions.

3. Observations on Systemic Reactions Induced by Injections of Mold Extracts.—Attacks of asthma or hay fever were induced in some patients by testing with mold extracts or by the injection of mold extracts for the purpose of hyposensitization. Some patients who had been injected simultaneously at separate sites with mold and other extracts developed systemic reactions to treatment. When under these circumstances the maximum local reaction occurred at the site of the mold injection, this was considered evidence that the latter caused the symptoms. When such evidence was not conclusive, the injection of mold extract alone was repeated at a subsequent sitting and the constitutional effect observed. In some instances we were unwilling to risk the induction of reactions a second time, and these patients were excluded from the study.

Of 526 allergic patients skin tested to molds, thirty-three had exacerbation of symptoms or systemic reactions after mold injections. Twenty-two of these developed symptoms following testing with mold extracts. Six of the twenty-two patients also developed symptoms after one or more mold treatments. Most of these reactions were mild in character, and in many instances no treatment was necessary. Eleven other patients developed symptoms after one or more mold treatments.

Of the twenty-two patients developing symptoms following mold testing, six experienced attacks of asthma within a few minutes. Several patients received small doses of adrenalin immediately, and one required 9 minims of adrenalin in three doses within a few minutes before the attack was relieved. One woman experienced her first attack of *status asthmaticus* the night following a skin test with an extract of *Aspergillus* isolated from her sputum. The attack persisted for several days. Subsequently she was treated with this extract in a dilution of 1:1,000,000 and experienced, for the first time in two years of treatment, marked alteration of symptoms. Five patients developed hay

TABLE II

PASSIVE TRANSFER EXPERIMENTS WITH SERUM FROM PATIENTS GIVING POSITIVE SKIN TESTS WITH MOLD EXTRACTS

PATIENT	A. C. 1 51			T. H. 2 177			R. P. 3 75c			H. P. 4 77c			E. S. 5 281			E. D. 6 25c			M. G. 7 38c			D. P. 8 280		
MOLD EXTRACT	DIRECT SKIN	RECEPTOR A1	RECEPTOR D4	DIRECT SKIN	RECEPTOR A1	RECEPTOR C3	DIRECT SKIN	RECEPTOR E5	RECEPTOR C3	DIRECT SKIN	RECEPTOR G7	RECEPTOR E5	DIRECT SKIN	RECEPTOR A1	RECEPTOR D4	DIRECT SKIN	RECEPTOR J9	RECEPTOR A1	DIRECT SKIN	RECEPTOR J9	RECEPTOR A1	DIRECT SKIN	RECEPTOR A1	RECEPTOR C3
Alternaria	++++[4]	+++[4]	+[2]	++[3]	++[5]	++[5]	+++	++	+++[aa]	++	+	?	+++[4]	++[5]	++[4]	±	—	○						
Hormodendrum	++++[3]	++[3]	+[2]				+	++[aa]	±	+[2]	++[2]	+[a]	+	○	+	+[3]	+[aa]	+	++	±[aa]	+			
Aspergillus																								
fumigatus							++	++	+	+++[2]	+[2]	+				±	?	○				+	+	±
ochrochus	++	+	○													±	+[a]	○	+	+[a]	○	?	?	+[a]
flavus				++	+	±	+	+	—				+	?	—	±	±	○	—	—	○	?	○	○
niger W 45																+	—	○				+++	++	+
Mucor																			++	±[a]	?			
Monilia sitophilia							+	+[a]	++															
Penicillium rubrum							+++	+	+	+	○	++												
Cephalothecium roseum	+	—	○	++	++	++				+	○	++[a]							++	+[aa]	+	+	±	—
Rhizopus	++	++	—													++[2]	+	○						
Dust	++	○	+[aa]	++	○	++[aa]	++	++[aa]	++[aa]	+++	++	○	+	○	+[aa]	++	○	○	++++	○	○	±	○	?
Ragweed	+	○	—	++	○	++	+++	+++	+++	+	±	±	+	○	—	++	○	○	+	○	○	—	○	?

TABLE II—CONT'D

PATIENT	C. R.* 9 1x			H. M. 10 68c			E. B. 11 10c			C. M. 12 232			M. P. 13 277			P. H. 14 50c			L. G. 15 39c			J. H. 16 48c			W. F. 17 30c			H. P.* 18 275	
MOLD EXTRACT	DIRECT SKIN	RECEPTOR A1	RECEPTOR D4	DIRECT SKIN	RECEPTOR F6	RECEPTOR G7	DIRECT SKIN	RECEPTOR E5	RECEPTOR C3	DIRECT SKIN	RECEPTOR A1	RECEPTOR C3	DIRECT SKIN	RECEPTOR C3	RECEPTOR L11	DIRECT SKIN	RECEPTOR G7	RECEPTOR D4	DIRECT SKIN	RECEPTOR G7	RECEPTOR F6	DIRECT SKIN	RECEPTOR E5	RECEPTOR C3	DIRECT SKIN	RECEPTOR L11	RECEPTOR M12	DIRECT SKIN	RECEPTOR H8
Alternaria	....	....	....	++	±	?	+	—	±	+	±	—	+4	+	+	+	±	—	....	....	....	+	±	?	....	....	....	○	±3
Hormodendrum	....	....	....	....	....	....	±	—	?	....	....	....	+	○	—	++	±	....	±	?	○	+	+a	?	++	±aa	+aa	○	+aa
Aspergillus																													
fumigatus	○	+2	+	....	....	....	....	....	....	++	+	—	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
ochrochus	○	±	+	....	....	....	....	....	....	....	....	....	++	++	++	++	+aa	+a	....	....	....	....	....	....	....	....	....	....	....
flavus	○	±	?	....	....	....	....	....	....	....	....	....	++	+	±aa	....	....	....	....	....	....	+	?	?	....	....	....	....	....
niger W 45	○	+aa	±aa	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	○	+aa
Mucor	....	....	....	++2	++2	++2	+	—	?	....	....	....	....	....	....	±	±	—	....	....	....	....	....	....	+	±	—	....	....
Monilia sitophilia	....	....	....	++	?	+	+	+	+a	....	....	....	....	....	....	+	±a	±	—	?	?	+	+a	±	+	?	±a	....	....
Penicillium rubrum	....	....	....	++	+	+	....	....	....	....	....	....	....	....	....	....	....	....	+	—	—	+	?	±	....	....	....	....	....
Cephalothecium roseum	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	±	+a	±	....	....	....	+	?	±a	....	....
Rhizopus	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	○	+aa
Dust	±	○	?	+	?	++	++	+aa	+	++	○	+++aa	+	++	+++	++	++	++aa	+	++	+	+++	○	±	+	+++	+	○	○
Ragweed	±	○	?	+	±a	—	+	—	—	+	○	+++	++	+++	++++	++	±a	+++	++	++a	±	?	?	○	?	—	—	○	○

* Patient did not have direct tests due to severe asthma.

○, Not tested; ±, slight reactions; ?, doubtful reactions; a, control equal to a ± reaction; aa, control equal to a + reaction.

2-3-4-5 indicates reagins transferable on 2-3-4-5 strains respectively.

Note: Patients 1 to 13 inclusive, successively transferred reagins to nonallergic subjects; patients 14 to 18 failed.

TABLE III

SYSTEMIC REACTIONS TO INJECTIONS OF MOLD EXTRACTS EMPLOYED IN SKIN TESTING AND TREATMENT

PATIENT	AGE (YRS.)	DIAGNOSIS	SYMPTOMS	INDUCED BY	COMMENTS	LARGEST SKIN REACTORS
W. B. 35	10	Hay fever, asthma, P., 6 years	Hay fever in a few minutes; adrenalin, 1½ minims	Mold tests†	Nasal obstruction after several treatments relieved when amount of mold extract was reduced	*Rhizopus*
W. B. B. 40	52	Asthma, F.	Asthma immediately; adrenalin	Mold tests*		*Alternaria* and *Helminthosporium*
C. B. 49	42	Asthma, F., P.	Severe attack of asthma	Alternaria test		*Alternaria* and *Helminthosporium*
A. C. 51	58	Asthma, F., 23 years	Itching and areas of dermatitis generalized after tests and scattered day after tests	Mold tests*	Asthma all of July, 1939, first time other than fall trouble; positive provocative tests; reagin present	5 *Alternaria*
H. C. 79	27	Asthma, F., P., 21 years	Asthma in a few minutes; adrenalin, 3 minims	Mold tests*		*Mucor* and *Alternaria*
E. D. 25c	50	Asthma, P., years	Asthma immediately; adrenalin, 9 minims	Mold tests	Severe reaction; reaction to provocative test	*Hormodendrum* and *Rhizopus*
H. G. 30	35	Hay fever, P., 1½ years	Unusual nasal obstruction continued one day	Mold tests		3 *Aspergilli and Hormodendrum*
M. G. 38c	25	Hay fever, P.	Faint, pale, right in chest in a few minutes; adrenalin	Mold tests*	Reaction to provocative test; reagin to *Hormodendrum*	*Hormodendrum*
J. G. 55	4	Hay fever, P., 2½ years	Asthma in a few minutes; adrenalin	Mold tests	Severe asthma in July and August, 1939	*Alternaria*
M. G. 140	30	Hay fever, P.	Hay fever in a few minutes; adrenalin	Alternaria test†	Severe hay fever and slight asthma in July and August, 1937; relief from *Alternaria* 1:100,000	5 *Alternaria*

W. H. 41c	47	Asthma, P., 1 year	Sneezing	Mold tests	Doubtful provocative test	*Alternaria*
C. H. 192	11	Asthma, P., 5 years	Headache, dizziness, pallor, nausea in few minutes; adrenalin	Mold tests		*Alternaria* and *Hormodendrum*
L. W. H. 222	55	Hay fever, asthma	Unusual hay fever for one day	Mold tests	Few other skin reactions	*Alternaria* and *Penicillium humica*
M. M. 266	29	Hay fever, urticaria, headaches	Hay fever in a few minutes; adrenalin, 1 minim; headache	Mold tests, 7 Aspergilli	Severe hay fever July 24, 1939, high *Alternaria* day	5 *Alternaria*
H. N. 270	49	Hay fever, arthritis	Nasal obstruction in a few minutes	Mold tests		*Cephalosporium*
E. S. 338	30	Asthma, P., 8 months	Asthma day following	Mold tests	Large, angry, delayed skin reaction at time of symptoms	*Penicillium rubrum*, *Alternaria*, and corn smut
C. M. T. 373	48	Asthma, P., 41 years	Nasal obstruction for several hours	Mold tests		
C. V. 383	46	Asthma, P., 30 years	Her first attack of status asthmaticus began night of test; continued for days	Test autogenous, Aspergillus	Improved for first time under treatment 1:100,000	
M. W. 388	14	Hay fever, P., 2 years	Increased hay fever in a few minutes; adrenalin	Mold tests†		5 *Alternaria*
E. R. W. 413	50	Hay fever, urticaria, 6 years	Severe generalized urticaria	Mold tests*		*Monilia sitophilia* and *Helminthosporium*
N. Y. 422	30	Hay fever	Hay fever	Mold tests		*Monilia sitophilia* and *Hormodendrum*
W. A. B. 43	45	Hay fever, F.	Severe hay fever	Mold treatments	No relief from high preseasonal ragweed treatment. After molds were added, she improved two or three times, then developed large local reactions and became worse. Patient went to bed for two weeks	*Monilia sitophilia* and *Helminthosporium*

TABLE III—CONT'D

PATIENT	AGE (YRS.)	DIAGNOSIS	SYMPTOMS	INDUCED BY	COMMENTS	LARGEST SKIN REACTORS
E. L. C. 80	44	Asthma, P., years	Asthma repeatedly	Mold treatments	Asthma recurred after mold treatments so often she discontinued them	2 *Hormodendrum* and *Aspergillus ochrochus*
V. D. 102	9	Asthma, P., 5 years	Asthma repeatedly in a few minutes; adrenalin	Mold treatments		4 *Alternaria*
J. C. H. 160	39	Hay fever	Hay fever within two hours on one occasion	Mold treatments	Several times mold treatments were reduced with improvement	*Hormodendrum*
R. H. 167	20	Hay fever, P	Hay fever in a few minutes several times	Mold treatments	Severe hay fever in July and August, 1939; marked reaction provocative test	*Aspergillus niger* W 45 and *Mucor*
T. J. H. 177	43	Hay fever, asthma, F.	Attack of urticaria and asthma on several occasions within a few minutes to two hours	Alternaria treatments	Hay fever July 24, 1939, a high *Alternaria* day; reagins for all Alternaria	5 *Alternaria*
H. P. 283	42	Hay fever, F. headaches	Severe headaches repeatedly in a few hours	Mold treatments	Refused treatment except ragweed because of repeated headaches	*Penicillium notatum*
G. P. 282	10	Hay fever, summer, 5 years	Hay fever in one or two hours	Mold treatments		2 *Alternaria* and *Hormodendrum*
E. S. 281	39	Hay fever, P. 3 years	Hay fever, tight in chest in an hour	Alternaria treatment	Reagins for all *Alternaria;* positive provocative test	5 *Alternaria*
T. T. 2x	25	Hay fever, weeks	Hay fever increased in one to two hours	Mold treatments	Hay fever disappeared when molds reduced	*Cephalothecium roseum*
M. T. 369	27	Asthma, P.	Asthma immediately several times	Mold treatments	Begged to have molds left off; unpleasant reactions ceased	*Hormodendrum, Aspergillus fumigatus,* and *Alternaria*
R. W. 403	27	Hay fever, spring, years	Hay fever	Mold tests		*Helminthosporium, Penicillium rubrum,* and *Penicillium humica*

*Reacted also to one or more treatments.
†Reacted repeatedly.
P., Perennial; F., fall.

fever within a few minutes following the mold tests, and one, a child, became ill with faintness and nausea within a few minutes. The nine other patients who experienced systemic reactions following testing developed typical allergic symptoms although appearance of their symptoms was delayed for several hours.

Among the patients who developed symptoms following mold treatment, three experienced asthmatic seizures within an hour or two, and one repeatedly developed asthma the night of treatment. Seven had hay fever within one to four hours after treatment, and one patient suffered repeatedly from severe headache the day of treatment. The headache did not recur when mold treatment was discontinued.

4. Provocative Tests.—Attempts were made to reproduce symptoms of asthma and hay fever by applying mold spores or concentrated mold extracts directly to the mucous membranes of the nose and throat. Mold extracts diluted 1:20 were sprayed into the nose and throat or applied directly to the nasal mucous membrane on a pledget of cotton. Mold spores were conveyed to the mucous membrane by a dry cotton swab on an applicator. A sterile swab was first rolled over a pure culture of the mold in a test tube and then was inserted just inside one nostril. Unless irritation ensued, the dry spore or wet extract applications were left in contact with the mucosa for fifteen minutes. Patients who developed positive reactions were retested at subsequent sittings with control swabs of cotton free from mold spores or mold extracts. Many with delayed or slight reactions were tested repeatedly with both molds and controls. Patients with active irritation or rhinitis were observed to react to any substance brought into contact with the mucosa and therefore were not tested until they had become free of symptoms. Every effort was made to prevent patients from recognizing the nature of the control tests. For these tests forty-nine consecutive clinic patients and twelve private patients were employed, all of whom gave positive skin tests to molds.

Twenty-two of sixty-one patients developed symptoms of allergic disease when spores or extracts were applied to the mucous membranes of the nose and throat (Table IV). In these patients *Hormodendrum* spores or extracts produced symptoms when applied to the mucosa in twelve patients; *Mucor* spores or extract produced symptoms in five patients, *Alternaria* in four patients, *Monilia sitophilia* and *Cephalothecium roseum* each in two patients, *Aspergillus fumigatus, Aspergillus ochrochus,* and *Penicillium rubrum* each in one patient.

Four patients developed frank attacks of asthma, one immediately, one after one or two hours, and the remaining two the same day. Most patients developed mild symptoms of hay fever beginning in about fifteen minutes and lasting from a few minutes to several hours. A few of the patients required adrenalin for relief. Twelve patients gave doubtful reactions and were not included in the list of positive reactors.

TABLE IV

PROVOCATIVE TESTS. MOLD SPORES AND EXTRACTS APPLIED DIRECTLY TO MEMBRANES OF NOSE AND THROAT

PATIENT	AGE (YEARS) AND SEX	DIAGNOSIS	SKIN TESTS		SPORES OR EXTRACTS PRODUCING NO REACTION	SPORES OR EXTRACTS PRODUCING REACTIONS	SYMPTOMS PRODUCED	CONTROL TEST
			DIRECT	PASSIVE				
E. B. 4c	42F	Rhinitis	+			*Cephalothecium roseum* spores	30 min.: Throat felt tight and swollen; patient dizzy; rhinorrhea for three to four hours	Negative
D. B. 5c	23F	Hay fever			*Aspergillus ochrochus* spores	*Mucor* spores	15 min.: Sneezing, aching of antra, membrane swollen None	Negative
P. B. 6c	47F	Asthma	+			*Aspergillus fumigatus* spores	30 min.: Faintness, dizziness, and nausea for one to two hours	Negative
			+			*Aspergillus ochrochus* spores	15 min.: Eyes itching and burning, nasal mucosa swollen	Negative
			+		*Cephalothecium roseum* spores		None	
E. B. 10c	24F	Hay fever	+	−−		*Mucor plum.* spores	15 min.: Sneezing, rhinorrhea for one day	Negative
			+	++		*Monilia sitaphilia* spores	15 min.: Lachrymation, sneezing, and rhinorrhea	Negative
					Corn smut extract		None	
R. C. 1xc	21F	Asthma	+			*Hormodendrum* extract spray	Few hours: Severe asthma first time in weeks	Negative
			+		*Alternaria* extract spray		None	
J. C. 18c	20F	Hay fever and asthma	++			*Hormodendrum* extract	15 min.: Sneezing and rhinorrhea	Negative
			++			*Mucor plum.* spores	15 min.: Lachrymation, sneezing, and rhinorrhea. Nasal obstruction followed	

E. D. 25c	50F	Asthma	+	+		*Hormodendrum* extract spray	Immediate asthma; adrenalin given	
M. G. 38c	25F	Hay fever	++	+		*Hormodendrum* extract 1:1000	5 to 10 min.: Pale, faint, tight in chest	Negative
I. G. 40c	62F	Hay fever	+			*Hormodendrum* spores	20 min.: Burning of eyes, and nose	Negative
						Hormodendrum extract spray	15 min.: Burning of eyes. 30 min.: Coughing and headache. Nasal obstruction all week, each time	
J. H. 48c	22M	Asthma and hay fever	+			*Hormodendrum* spores	30 min.: Rhinorrhea and sneezing	Negative
R. M. 60c	61M	Asthma	+			*Cephalothecium roseum* spores	15 min.: Sneezing, rhinorrhea, and nasal obstruction	Negative
L. M. 63c	59F	Asthma and hay fever	+			*Hormodendrum* extract	30 min.: Headache, eyes burning, and mild asthma	Negative
			+		*Penicillium rubrum* spores		None	
J. P. 73c	18M	Asthma	++			*Hormodendrum* extract	15 min.: Rhinorrhea which continued for some time	Negative
A. P. 76c	26F	Hay fever	+			*Hormodendrum* spores	15 min.: Rhinorrhea	Negative
H. P. 77c	50F	Asthma	++	+		*Alternaria* spores	Asthma in one to two hours. Adrenalin given	Negative
						Alternaria extract	20 min.: Rhinorrhea. Same night severe attack of asthma, continued twenty-four hours	
			+++	+	*Aspergillus fumigatus* spores		None	
			++	0	*Aspergillus ochrochus* spores		None	
			+	++	*Hormodendrum* spores		None	
			+	++	*Helminthosporium*		Slight	

TABLE IV—CONT'D

PATIENT	AGE (YEARS) AND SEX	DIAGNOSIS	SKIN TESTS		SPORES OR EXTRACTS PRODUCING NO REACTION	SPORES OR EXTRACTS PRODUCING REACTIONS	SYMPTOMS PRODUCED	CONTROL TEST
			DIRECT	PASSIVE				
C. S. 87c	37F	Hay fever	++			*Monilia sitophilia* spores	30 min.: Sneezing and coughing	
G. W. 101c	39F	Asthma	+			*Monilia sitophilia* spores	30 min.: Sneezing and coughing	Negative
			+			*Mucor plum.* spores	10 min.: Weeping, rhinorrhea, and sneezing	
						Hormodendrum extract	5 min.: Sneezing, rhinorrhea lachrymation. 10 min.: Head ache	
E. Y. 102c	39M	Hay fever and asthma	+			*Hormodendrum* extract	15 min.: Rhinorrhea, nasal obstruction. Swelling of eye, nose and mucosa	Negative
						Hormodendrum extract spray	Nasal obstruction	
R. P. 75c	27M	Asthma and hay fever	++	++		*Alternaria* extract 1:100	6 min.: Lachrymation, sneezing, and rhinorrhea for one hour	Negative
A. C. 51	58F	Asthma	++++	+++		*Alternaria* extract	10 min.: Sneezing, rhinorrhea nasal obstruction for one hour	Negative
						Hormodendrum extract	6 min.: Rhinorrhea, nasal obstruction; adrenalin administered	
R. H. 167	20F	Hay fever	+			*Mucor circ.* extract	10 min.: Sneezing, obstruction and rhinorrhea. Hay fever for one week	Negative
E. S. 281	39F	Hay fever	+++	+++		*Alternaria* extract	15 min.: Rhinorrhea, nasal obstruction, lasted several hours	Negative

Twenty-two patients showed positive reactions to provocative tests for one or more molds with negative controls; six of these patients also gave negative tests to one or more molds.

Six of the twelve had reactions to the control tests, and in six the reactions were of such slight degree or duration as to be of questionable significance. Twenty-seven patients exhibited no reaction to the application of mold spores or mold extracts to the nasal mucosa.

DISCUSSION

The four approaches used in this attempt to determine whether clinical sensitivity existed and was of practical significance in a group of patients who gave positive skin tests to other and commonly recognized allergens yielded results which we believe to be important.

Many patients who gave one or more positive skin tests to molds have been treated with mold extracts. Most of these have responded well, but because of the multiplicity of sensitivity, and the multiplicity of antigens used, their specific response to mold therapy could not be evaluated and they were not included in the group with the seventeen patients whose improvement was marked after the addition of mold extracts to their treatment.

The positive skin tests to mold allergens which could be confirmed by the indirect method of testing in thirteen of eighteen patients were considered as affording strong evidence of true mold sensitivity. Our findings indicate that results with the indirect method may be predicted to some extent by the degree of reactivity to the allergen of the serum donor's skin.

It is our impression that a few patients other than those reported, thirty-three, or 7.3 per cent, of 526 patients tested, exhibited allergic symptoms due to mold testing or treatment, but, because of multiple factors involved, the evidence did not seem conclusive.

Of the twenty-two patients who reacted symptomatically to direct applications of one or more molds to the nasal mucous membrane, eight gave positive results with passive transfer tests. The blood sera of these eight patients, with one exception, contained demonstrable reagins for the mold producing symptoms. The serum of one patient contained reagins for *Monilia sitophilia* but not for *Mucor*, although exposure to either mold resulted in allergic symptoms. The sera of the other members of the group also contained reagins for molds which did not produce symptoms. No reagin was demonstrated in the sera of patients with negative provocative tests. Only one patient with a slight or doubtful provocative reaction yielded transferable reagins. This patient, a man, reacted very slightly to the provocative *Alternaria* test, although his serum, when passively transferred to a control subject, induced positive skin reactions to five strains of this mold. The patient had been treated continuously with *Alternaria* extract for a period of nine months before a provocative test was made.

Eight of the sixty-one patients receiving provocative tests developed systemic reaction to mold injections. Six of the twenty-two positive

reactors to provocative tests and two of the twelve doubtful reactors developed these reactions. Thus a total of forty-nine patients gave positive reactions to provocative tests or mold injections. It is felt that positive clinical reactions to provocative tests or to injections of mold extracts constitute evidence of the presence of clinical sensitivity.

A comparatively large number of children appeared to be clinically sensitive to molds. Fifty-one, or 9.7 per cent, of our entire group of 526 patients were children. Eleven, or 25 per cent, of the group of midsummer sufferers and six, or 35 per cent, of the seventeen patients improving after the addition of molds to their treatment were children. Also, five, or 15 per cent, of the group of the thirty-three patients who developed systemic reactions following mold injections were children.

SUMMARY

1. Sixty-seven of a group of 526 patients gave histories strongly suggestive of mold sensitivity.

2. Reagins to molds were demonstrated by passive transfer in thirteen of eighteen patients skin-sensitive to molds. The size and intensity of the direct skin test was indicative but not diagnostic of the presence of reagins.

3. Thirty-three of 526 patients, or 7.3 per cent of the total number, developed systemic reactions to mold injections.

4. Provocative tests were made on sixty-one patients who were skin sensitive to mold, and twenty-two, or 36 per cent, of these gave positive provocative reactions, thus demonstrating the presence of clinical sensitivity.

5. Mold sensitivity is not uncommon and must be taken into account in any complete study of the allergic state.

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DISCUSSION

DR. HOMER E. PRINCE, Houston.—At the outset of her paper, Dr. Pennington very clearly showed that direct skin testing in suspected mold allergy is by itself an insufficient criterion for etiological diagnosis. I believe almost every honest investigator who has tested patients with mold extracts will admit that many reactions are obtained, the importance of which is often confusing. In the light of our present knowledge, there is a great temptation to regard mold seasons as indefinite, and variable quantities so confusing and overlapping the periods of incidence of better known allergens that, when the latter fail to explain our difficult patients' troubles, we are prone to seize upon any mold reactions as of etiological significance in the same manner that we regard many perennially occurring allergens as well as foods. Possibly another source of error in this connection is the titration with strong extracts in our search for reactions, until we are prone to accept purely irritative responses as etiological possibilities. On the other hand, we are constantly being impressed with the fact that fungus material is often perennially present and that in many environments, particularly in the middle and southern portions of the country, there is some constant atmospheric mold load which is subject to certain definite seasonal fluctuation. It would seem then that it becomes a matter of considerable importance for the allergist to be able to determine when he is confronted with a clinical mold problem.

In her approach to the problem of establishing molds as definite etiological factors in certain of her patients, Dr. Pennington has been conservative. Employing methods of approach familiar to all of us, she has shown that the sera from thirteen of eighteen selected patients contained skin sensitizing antibodies. This incidence of about 70 per cent of positively reacting patients having transferable antibodies suggests not only that her patients have been exposed to enough mold to initiate specific sensitization, but also that any symptoms encountered by these patients upon exposure to certain mold allergens might be specifically incited.

Figures dealing with the incidence of increased symptoms or systemic reaction following testing or treatment with any allergen might vary with individual investigators and different techniques. The fact that Dr. Pennington observed thirty-three instances of increased symptoms or systemic reactions in 526 patients skin-sensitive to molds, a percentage of 7.3, again suggests that in these patients sensitization to the allergens employed did exist. The fact that seventeen patients improved following addition of mold allergens to their therapy further suggests the specific etiological relationship, inasmuch as this was the only change made. Up to this point Dr. Pennington's evidence has not proved any clinical relationship of molds in respiratory allergic disease, but the evidence has been strongly suggestive. However, in those patients who had definitely proved molds in their local environment and who improved upon elimination of contact with these molds, it seems that etiological relationship becomes more irrefutable. This should emphasize to all of us the value of painstaking environmental surveys.

Dr. Pennington's technique of performing her provocative tests did not exactly reproduce the conditions of natural contact. Perhaps it would have been better had she been able to place these patients in a

closed room where mold spores were being agitated by an electric fan, but doubtless the small quantities of mold material with which she had to work precluded this type of experimentation. However, her control testing seems adequate, and she has carefully reported as negative all patients who reacted either questionably or to the control applications to the nasal mucosa. It is apparent that twenty-two of her sixty-one patients thus tested by exposure to mold allergens developed clinical manifestations from the short period of fifteen minutes. It is interesting that, in eight of these twenty-two patients studied by passive transfer, reagins were found to all the molds causing clinical reproduction of symptoms, except in one case. Unfortunately, all twenty-two of the patients reacting positively to provocative testing were not studied by the passive transfer method.

Dr. Pennington's observation that many patients sensitive by skin tests had trouble during periods of increased incidence of these molds is concrete and practical evidence of the importance of mold spores in her environment. I believe that the finding of thirteen reactors to *Hormodendrum* among the twenty-two positive provocative reactions indicates much more than a chance observation. I base this statement on the fact that fourteen months ago a survey that is still being conducted demonstrated that concentrations of *Hormodendrum* in the vicinity of Nashville are particularly high. If allergic individuals become sensitive to fungus materials, it would be only logical to assume that the incidence of this sensitization would be particularly high to a form as dominant as *Hormodendrum* seems to be around Nashville.

Dr. Francis M. Rackemann, Boston.—It seems to me that this paper is too important and too practical to pass over quickly. There are numerous things to say about it. The emphasis on the lack of correlation between the skin tests and the clinical history is, of course, very pertinent. Whether Dr. Pennington will say that molds in the air are different from molds in the house is another point.

Up to about two years ago, there were only ten cases in the literature in which the relation of mold to asthma could be definitely proved as by the inhalation of mold spores or the exposure of patients to a powder derived from the dried pelts of mold cultures. Dr. Pennington has added more cases to that list.

I have one question, Dr. Pennington. In making your provocative tests, you could demonstrate that certain molds could produce the symptoms. Were those same patients tested with other molds at the same or another time? In other words, could you show that the provocative test was a specific test?

Dr. Pennington.—Patients were given provocative tests in several instances for several molds; in each case they were tested only for molds which had given positive skin reactions. Several gave positive provocative tests to one mold and not to others, and some gave positive reactions to two or three molds. All the data are given in a table which is too lengthy to be put on a slide.

Society Proceedings

THE AMERICAN ASSOCIATION FOR THE STUDY OF ALLERGY

ANNUAL MEETING, NEW YORK, N. Y., JUNE, 1940

Electrocardiographic Changes in Bronchial Asthma and Their Significance. JOSEPH HARKAVY, M.D., and ALFRED ROMANOFF, M.D., New York, N. Y. (Abstract.)

This study includes observations made on fifty patients suffering from classical symptoms of bronchial asthma. In the course of the asthmatic paroxysms twenty patients showed electrocardiographic changes which involved the auricular complexes, the ventricular complexes, or both. A review of the constitutional background of these indicated the presence of personal or family history of allergy in only five. Sinus infection proved to be the major exciting agent in eighteen of the cases, and in eight of these, food and inhalant sensitivity including pollen was likewise demonstrable. The provocative factors in the remaining two cases were not determined. The duration of asthma varied from eight months to a maximum of thirty-four years. The youngest patient in this series was 18, and the eldest, 60 years of age.

Nine of the twenty patients showed reversible electrocardiographic changes returning to the normal state with the termination of the asthmatic paroxysms, and five presented irreversible changes. The latter were accounted for by permanent myocardial changes associated with the persistence of the asthmatic state in three patients and the development of chronic pulmonary emphysema of such extent as to lead to the production of lung cysts in two. The other six patients could not be classified as to reversibility or irreversibility because of inadequate follow-up studies.

Analysis of the electrocardiographic changes revealed auricular extrasystoles in one case, the presence of high P waves only, in six, and augmented P wave associated with QRS and T-wave deviations in eight of the twenty patients. The remaining five cases presented alterations confined to QRS and T waves alone. The QRS complex changes were characterized by slurring, notching, or low voltage, while the T-wave abnormalities were low, isoelectric, or inverted.

The development of the unusually high P waves in fourteen of the twenty cases was interpreted as the result of increased intra-auricular tension, secondary to hypertension of the lesser circulation, brought about by spasm and edema of the pulmonary vessels similar to rabbit

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anaphylaxis. Support for such a concept was found in six of the patients who coincidentally with the electrocardiographic changes developed interstitial pulmonary infiltrations demonstrable by x-ray examination. These were interpreted as being due to allergic inflammatory reactions in the vessels of the lungs associated with the increased tension in the pulmonary circuit. The lesions in the lungs together with the electrocardiographic abnormalities disappeared with the conclusion of the asthmatic seizures, indicating their interrelationship as well as their reversibility. Microscopic studies of the lungs of three autopsied cases recently reported by one of us (J. H.) showed similar infiltrations on x-ray films and disclosed thickening of the pulmonary vessels as well as arteritis with eosinophilic infiltration.

The high P waves in the remaining eight cases having negative x-ray findings in the lungs also returned to normal with the cessation of the asthmatic state. This reversion of the high P wave with the termination of the asthmatic seizures in both groups was considered a sequence to the reduction of the tension in the pulmonary circulation. The temporal relationship suggested a common pathogenesis.

The QRS and the T-wave changes were considered to be due to coronary artery spasm and edema similar to that in the pulmonary vessels, leading to local myocardial ischemia and insufficiency. This interpretation is offered contrary to the one held by some, to the effect that the general respiratory anoxemia accompanying the asthmatic attacks is responsible for such changes. Whereas the contributory role played by respiratory anoxemia in causing electrocardiographic alterations may not be excluded, this cannot be accepted as the only explanation for the abnormalities observed in certain cases of bronchial asthma, especially in view of the conflicting evidence by different investigators as to the degree of existing anoxemia in this disease. In one of our cases, for example, chosen because of the presence of emphysema, lung cysts, and cyanosis, studies of the oxygen tension of the blood in the presence of irreversible QRS changes showed it to be 18.6 volumes per cent and the arterial carbon dioxide content 44 volumes per cent. Moreover no electrocardiographic changes were found in thirty of the fifty cases in this series during similar asthmatic paroxysms. This would suggest that the patients with electrocardiographic changes had the coronary vessels as an additional shock organ. In those instances in which the reaction in the coronary vessels subsided with the termination of the asthmatic attack, the restoration of the normal coronary flow was succeeded by normal electrocardiographs.

Clinical evidence of the fact that respiratory anoxemia alone was not responsible for the changes in the ventricular complexes was illustrated by two of the cases in this series in which the deviations in the ventricular complexes appearing during the asthmatic paroxysm persisted in the absence of cyanosis and asthma, at a time when the pulmonary manifestations gave way to allergic symptoms in other shock organs.

In one case the asthmatic seizures receded with the development of an eosinophilic peritonitis, arteritis of one of the mesenteric vessels with thrombosis and infarction of the gut, as well as polyneuritis. In a second patient the asthma was replaced by acute pericarditis, joint manifestations, and urticaria. Whereas the persistence of the electrocardiographic changes could be attributed to the pericarditis, the significant point is the shift of involvement from the pulmonary tissues to the cardiovascular structures, under the same allergenic stimulation. The electrocardiographic changes in both of these patients reverted to normal with recovery and the disappearance of the manifestations in these new shock organs. In a third patient, who developed irreversible electrocardiographic alterations with enlargement of the right and left ventricles demonstrable by x-ray, progressive hyperergic vascular disease of the coronary vessels was suggested by the biopsy of a nodule present in the conjunctiva, which showed perivascular eosinophilic infiltrations. Such changes are similar to those observed in the early stages of periarteritis nodosa.

DISCUSSION

DR. HARRY L. ALEXANDER, ST. LOUIS.—Dr. Harkavy has presented a very interesting and I think a new finding in a large series of cases such as this; namely, during an attack of asthma, there are electrocardiographic changes which disappear when the asthma disappears.

Now the interpretation of this phenomenon is very difficult to prove, as Dr. Harkavy has stated, and so becomes speculative. He has indicated that to his satisfaction he has ruled out certain conditions including anoxemia. He infers, therefore, that the changes in the heart are due to allergy of the heart induced by the same allergen that in the bronchi causes asthma. I think that is a very interesting thought, but I do not feel that I can quite accept it with the readiness with which he presented it because the changes that he showed in the RST complexes are so characteristic of mild anoxemia that I think that point must be ruled out before we can give his interpretation to it.

I quite agree with Dr. Harkavy's interpretation of change in the P wave. I think that is quite possibly due to a hypertension of the pulmonary circulation.

Now anoxemia does occur in most patients with asthma because they are cyanotic to begin with and cyanosis is merely a visual expression of unsaturation of arterial blood. Notoriously during an attack of asthma, there may be a great unsaturation. The case Dr. Harkavy mentions had 22 volumes per cent oxygen capacity, 18.6 oxygen content, which is about 10 or 15 per cent unsaturation; this is sufficient to give electrocardiographic changes. I would ask Dr. Harkavy, therefore, whether an anoxemia must not be ruled out in his cases before one accepts his idea that this may be due to allergy of the heart.

Concerning this latter conception, I wonder if this could not be better established by considering a series of cases of gastrointestinal allergy or vasomotor rhinitis and taking the electrocardiograms before and after season, or before and after abdominal attacks. This would completely rule out all the severe dynamic changes which take place in the thorax

during asthma. If the heart then reacted to these allergens by electrocardiographic changes, I think it would be a little more convincing that these changes are due to allergy of the heart.

Dr. Leo H. Criep, Pittsburgh.—I think this is a very valuable contribution because we frequently are in doubt in dealing with a patient with bronchial asthma as to what role the heart plays in producing symptoms. Especially is that so in older people where the presence of arteriosclerotic heart disease is to be considered. The question frequently arises in these instances to what extent are the patient's respiratory difficulties due to cardiac insufficiency and to what extent are they due to an allergic asthmatic condition. The electrocardiographic changes which were shown are somewhat different from those which we presented some few years ago. At that time we reported electrocardiographic changes observed in a series of patients with bronchial asthma and showed that there were changes in rhythm occurring during attacks of bronchial asthma; we were unable, however, to demonstrate electrocardiographic evidence of such advanced heart muscle changes as those shown in this presentation.

It must be remembered that changes seen in the T wave in Lead II are not always reliable. The T II changes frequently are transitory and need not necessarily indicate grave myocardial lesions.

The last case showed deep inversion of the T wave in Lead I and the patient died; the tracing was very suggestive of infarction. I am anxious to know whether this patient showed any evidence of myocardial infarction at post-mortem examination.

A few years ago we reported definite electrocardiographic changes in guinea pigs in anaphylactic shock. The same findings were demonstrated in guinea pigs during acute asphyxia. These electrocardiographic findings were indistinguishable from those in acute myocardial infarction in the human being, but we were never able to find similar changes in bronchial asthma.

Dr. J. Alexander Clarke, Jr., Philadelphia.—Just a practical point, and that is estimating right ventricular preponderance. If a person has a disease of the heart, hypertension, for example, which would cause hypertrophy of the left heart, he may also have asthma causing hypertrophy of the right heart, and the net result will be no preponderance on either side. It would be a good point for cardiologists to remember when they see a person with hypertension, cardiac hypertrophy, and pronounced dyspnea. If the electrocardiogram shows no preponderance, the lack of preponderance might be due to hypertrophy of the right heart from the asthma which is causing the dyspnea.

Dr. Ralph H. Spangler, Philadelphia.—I should like to ask Dr. Harkavy one question. Are any interpretations possible with the use of the electrocardiograph, or do cardiologists have any means available to determine whether it is the pulmonary circulation or the cardiac circulation that is affected when the amplitude of the P waves is increased in asthmatic or other patients?

Dr. Harkavy.—I am fully aware of the shortcomings in my argument, and I am sorry that I did not have a chance to read my paper in its entirety. I realize that the explanation that I have given for the findings in my cases is based on circumstantial evidence. This is due to

the fact that it is impossible to measure the tension of the lesser circulation directly, unless you kill the patient by putting a cannula into the auricle.

I am also cognizant of the fact that anoxemia may be an important factor in producing electrocardiographic changes and that different individuals vary in their sensitivity and response to oxygen want. In other words, small amounts of oxygen want may produce electrocardiographic abnormalities in some patients, whereas in others no such changes may result. Nevertheless it is significant, although not conclusive, that, in spite of variations in the degree of anoxemia present during asthmatic attacks in the fifty cases studied, only twenty patients showed electrocardiographic changes, whereas the remaining thirty, although manifesting similar paroxysms, failed to show such disturbances. Moreover, in the average asthma patient one rarely finds electrocardiographic abnormalities. It may be assumed, therefore, that the varying amounts of anoxemia accompanying the asthmatic paroxysm in different individuals are not as a rule followed by electrocardiographic abnormalities. What other explanation can therefore be given to account for the changes observed in our particular group? A clue to this may be found in the fact that some of the patients under observation developed signs and symptoms suggestive of a diffuse vascular involvement. The electrocardiographic changes may consequently represent hyperergic, reversible and irreversible vascular reaction in the heart, initiated by the same agents responsible for the asthmatic attacks.

Dr. Alexander's suggestions for obtaining additional proof for such a contention by investigating the electrocardiograms in hay fever and gastrointestinal patients is excellent, providing such patients, similar to the ones reported, also have the heart as a shock tissue. In this connection I should like to remind you that in 1938 I reported a group of patients with paroxysmal tachycardia and vasomotor rhinitis following food hypersensitiveness. In 1939 I presented another series of eight cases with manifestations of asthma, pulmonary infiltrations with eosinophilia and electrocardiographic changes on the basis of hyperergic vascular disease; one of the patients in this group did not have asthma, although she developed electrocardiographic disturbances in association with the pulmonary infiltrations similar to the others. With the termination of the pulmonary lesions which were regarded as due to allergic reactions in the vessels of the lungs, the electrocardiograms returned to normal. It is apparent, therefore, that in the absence of asthma and of accompanying anoxemia the electrocardiographic changes must have been due to disturbances in the heart brought about by local influences which were but a part of a more generalized hyperergic vascular response. This, as you may remember, was proved by a biopsy of an area of hemorrhagic necrosis present in the thigh of this patient which showed perivascular infiltrations with eosinophilia. It may, however, be justly argued that in the final analysis vascular changes in the heart muscle, even of a hyperergic nature, which bring about alterations in the electrocardiogram are necessarily due to local anoxemia. Nevertheless it is important to establish that the latter is a secondary result of allergy rather than the primary effect of generalized oxygen want.

In answer to the question of Dr. Criep, the patient with marked inversion of the T wave had no infarction of the heart demonstrable at autopsy. He showed only slight hypertrophy of the right ventricle.

I wish to say that I am in full accord with Dr. Hensel, who warned against attaching too much importance to the electrocardiogram, in con-

trast to the clinical findings in the heart. I myself prefer to use laboratory data as a means rather than as an end in diagnosis. In the cases presented, however, we could never have obtained the desired information by physical examination of the heart alone. On auscultation these cases presented only tachycardia with a fairly good muscular sound. No other abnormalities were detectable. It is only in the more exaggerated cases where you get gross cardiac irregularities or other phenomena associated with cardiac insufficiency that physical examination is enough to establish the diagnosis of myocardial damage. The electrocardiograph was therefore of inestimable value in disclosing the myocardial abnormalities which we have reported.

DR. SPANGLER.—Auricular circulation, cardiac circulation—is there a method to determine which is predominant?

DR. HARKAVY.—It is impossible to determine the tension of the lesser circulation by any direct clinical methods. Under normal circumstances there is a reciprocal relationship between variations in the tension of the pulmonary circulation and the heart action. Up to a certain point increase in the tension of the lesser circulation may be compensated by increase in the heart rate. Beyond that, augmentation in the tension of the lesser circulation may be reflected in the heightened auricular pressure registered in the electrocardiograms by high voltage of the P waves. When this tension is further accentuated, it may be indicated in the increased venous pressure which can be measured manometrically. Thus it may be stated that the large P waves reflect the state of the pulmonary circulation, in the absence of endocardial or other well-established etiological factors.

If one accepts the fact that the heart is a shock organ, it may explain the various electrocardiographic changes in the ventricular components noted by us on the basis of hyperergic vascular reactions in the myocardium itself. As I previously stated, I have as yet insufficient proof that this is so in every instance studied. In view of our previous observations, however, and the similar phenomena presented by these cases we wish to direct your attention to a group of patients with bronchial asthma in whom the repeated association and disappearance of pulmonary and cardiac abnormalities concurrently with the asthmatic paroxysms suggest a common etiology and pathogenesis. This type of case may be differentiated from the usual variety of bronchial asthma by x-ray examination of the lung, electrocardiographic studies, and, frequently, high eosinophilia.

Anthropometry in Children: Progress in Allergic Children as Shown by Increments in Height, Weight, and Maturity. MILTON B. COHEN, M.D., and ROSS R. WELLER, M.D., Cleveland, Ohio, and STANLEY COHEN, M.D., New Orleans, La. (Abstract.)

This is a report of five cases with charts showing increments in height, weight, and maturity during the progress of the treatment of allergic manifestations.

These cases indicate that failure to make standard gains in weight is the first disturbance noted. The failure to make suitable weight gain is

followed by diminution in gain in height and in most severe cases in failure in increments of maturity.

The reports illustrate the value of anthropometry in recording progress in allergic children.

Allergy is only one of many conditions which may disturb the growth pattern. When careful study enables one to rule out conditions other than allergy, the developmental progress of a child can be used to determine the efficacy of the treatment of the allergy. No child who fails to achieve suitable and regular increments in height, weight and maturity can be considered healthy. Careful clinical study of the children who are lagging in development will usually disclose some chronic handicap, the removal of which will be followed by improvement in the developmental pattern and often by complete compensation.

DISCUSSION

Dr. Aaron Brown, New York.—Generally speaking, children with allergy fall into two classes. They are either obese or very, very much underweight, but they become normal eventually, as was shown on the charts, when they respond to specific treatment. One day two children with asthma walked into my office. They were both 9½ years old. One weighed 143 pounds; the other, 69 pounds. Four years later both were free of asthma. The former child weighed 175 pounds; the latter weighed 93 pounds. If the figures alone were given, they would not mean much, but, if one considers these in relationship to the parents, then they assume significance. Of course, the parents of the fat boy were big. The father weighed 300 pounds; the mother, a little over 200. The parents of the other child were thin and never weighed more than 115 pounds. When the father was 9½ years old, he presented the same picture as that little, thin, anemic-looking child, though he had no asthma.

So we must take all these facts into relationship because, after all, we do inherit a constitution and people are very short or they are tall depending on what their parents were, except that our children today are usually taller than their parents.

I think that this work is so valuable that I would suggest to Dr. Cohen that he send to interested allergists a summary of the tables of weights, heights, and maturity he wants listed so that we can study routinely our children along the same lines. If enough allergists do that, at the end of a year, probably 1,000 cases could be reported, instead of a few cases which are examples, and then we could draw proper conclusions as to any relationship between the allergic child and his development.

Dr. Harry S. Bernton, Washington.—If this method were applied to the study of tuberculous children who, likewise, would be suffering from a disease of long standing, would the end results be any different from those Dr. Cohen has achieved in allergic children?

Dr. J. Alexander Clarke, Jr., Philadelphia.—I want to second the importance of this work. I wonder if Dr. Cohen would have time to give us briefly the data on which he bases his maturation.

DR. BRET RATNER, New York.—I believe that Dr. Aaron Brown's suggestion might help us to gather valuable information. I would like to ask Dr. Cohen several questions. One is, does he believe that allergy that is improved will have an increased increment in weight, height, and maturation if nothing else is done to the child besides the allergic phase?

Does he not believe that the practice of giving vitamins and taking care of the child from a pediatrics standpoint is essential in all cases?

Then there is the question of maturation. We have followed about 1,500 children for maturation, and we find in the allergic patients, as we do in our general practice, that a certain proportion of them are immature by ossification maturation criteria. In these cases we have used thyroid as an accessory agent and believe that thyroid, vitamins, and the good resulting from the reduction of contact to allergens probably all play important roles. The presence or absence of allergy alone does not, in my opinion, control height, weight, or maturation. The child must always be viewed as a complex, growing organism. Allergy may retard such growth, but other factors play a potent role. The child must be viewed from many angles and treated as an entity and not as just an allergic patient.

DR. GEORGE PINESS, Los Angeles.—The paper should interest the allergist in adding to his observations of the allergic patient (particularly the allergic child) those factors suggested by Dr. Cohen. I am safe in assuming that most clinics do have some system of follow-up on their patients where nutrition and growth are concerned. In the average clinic it is most difficult to carry out the program suggested by Dr. Cohen. This type of research is particularly timely in a clinic such as Dr. Todd's at the Brush Foundation where main interest is that of growth and nutrition and the equipment lends itself to this work, but it would be impossible to follow the minute details in an average clinic where 100 to 150 children are seen per day. Such clinics are fortunate to have weights and heights recorded once a month.

Private practice offers a greater opportunity than the free clinic, but here again is added a difficulty in the inability to follow these patients over as many years as in the Brush Foundation. Dr. Todd has followed some of his cases over a period of fifteen to twenty years. I doubt whether this is possible in our private practice. We can, however, carry out the suggestions offered by Dr. Cohen insofar as the follow-up of weight, height, and general nutrition is concerned, but I doubt very much our ability to bring in figures such as Dr. Cohen presented today.

DR. GEORGE L. WALDBOTT, Detroit.—I should like to ask Dr. Cohen if he has any data on adults. He has been speaking of children only. If we consider changes in height, weight, bone density, and in the menstrual cycle of women which so frequently accompany the asthmatic state, one should think that adults might be a fertile field for this type of work.

DR. COHEN.—Of course, there is a family pattern. That is why one must deal with the increments. One takes the temperature of a pneumonia case. It is 103° F. The important thing is what happens to the temperature as the patient goes on with his pneumonia. The important

thing here is what changes occur during the period of observation. The increments determine prognosis.

These changes, Dr. Bernton, are due to disturbances or correction of disturbances in nutrition. After all, nutrition is the important thing. We have some evidence which will be published shortly that people with allergy, and particularly allergy mediated through the gastrointestinal tract, do not use food substances, vitamins, minerals, as well as do those who are allergic or do not have these active symptoms. We also know there are changes in minerals. Any illness may cause similar changes. I said in the beginning that these are not pathognomonic of allergy, but we must be doctors, and a child is not well so long as he does not progress properly.

Dr. Clarke, the data on maturation will be found in Todd, *Atlas of Skeletal Maturation*. In it you will find pictures of hands of boys and girls from 3 months to full adult development. The maturation estimations are done not on the basis of what Dr. Ratner is talking about, the appearance of centers of ossification, but on the relative amount of mineralization of the cartilaginous epiphysis and its general shape and the size.

I think, Dr. Ratner, that answers one of your questions. We have to be doctors, of course. That is what I am trying to emphasize. We cannot be just allergists. We do not treat a disease; we treat people who are sick. Otherwise I do not think we are practicing good allergy. Allergy is only the platform on which one does something for the patient.

I should like to point out something about ossification development because I think it is important and it may be confusing. The literature is full of statements that, when a child's hand shows missing centers, that child has thyroid disease. That is by no means true. The child may have thyroid disease. When a center which should appear, say, at 10 months does not come, then it means that something happened to the child that disturbed his development between 6 and 9 months and, therefore, he did not put in the center. Nature has a schedule for putting in centers. She expends her major energy in doing the things she should do. Now, if something happens which prevents development at a particular time, nature goes ahead with her schedule and ultimately will make up the deficiencies from reserve capacity, whatever that may be, one year or two years or five years later.

If one is to try to assess the maturation age on the basis of centers, one can do this only up to about 2½ to 3 years relatively well. The ones which are not in indicate that one can go back in the history and find out what was the matter with the child at the time this center should have come in but did not.

Dr. Piness, these things which I presented today are things which, in my opinion, are within the scope of the man in private practice. At the Brush Foundation there is a study so broad that no one could hope to do it; but out of these studies made by research organizations we ought to be able to pick those things which are useful in clinical practice, and I think these are useful. The most useful are the height and weight, and anyone can do them. The maturity is desirable and is within the scope of any of us who is willing to have a flat picture of the hand made and who will spend about six months learning to assess.

There is one more question to answer here, and that is Dr. Waldbott's about adults. The adult shows the end results of the impact on the constitution of vicissitudes. One cannot study growth in an adult; he has finished growing. The time to study these things is in childhood.

Whealing Response to Ultraviolet Light and the Histamine Theory of Allergic Reactions. HAROLD A. ABRAMSON, M.D., New York, N. Y. (Abstract.)

For the past three years a case of urticaria solare has been studied, and the following data may now be reported.

The region of spectral hypersensitiveness (in a 32-year-old woman patient) as manifested by whealing begins very close to 3700 A° and extends to lower wave lengths with increasing sensitiveness in the region where absorption occurs. This zone corresponds to the zone of response of the skin to pigmentation reported by Luckiesh and Taylor.[1] The syndrome, therefore, might be connected with the pigmentation process, although pigmentation may be induced in the usual way. The case studied by Blum and his associates[2] is probably another entity, for his case responded in the visible region.

Observations of the development of over 500 wheals after exposure to various sources of light have shown that pseudopods never form. The borders of the wheal always initially coincide exactly with the area irradiated, and the wheals last several hours if the stimulus is sufficient. Spread of the wheal is slight but uniform and is probably due to an increase in intradermal pressure caused by an excess of extravascular liquid. The nondevelopment of pseudopods is not in accord with the theory that a readily diffusible H-substance like histamine is liberated in the tissues subsequent to irradiation.

An irradiated zone of the skin which had many needle punctures was treated with wet cotton and the negative pole to electrically transfer histamine from the skin during the whealing process by reversed electrophoresis of histamine. A whealing substance was not isolated from the skin by this technique although histamine itself may under certain conditions be recovered in this way.[3]

A large area of the back was irradiated; this caused the formation of a wheal of approximately 200 square inches without any symptoms of histamine poisoning.

A histamine wheal may be readily formed (electrophoresis or injection) over an irradiated area of the skin which has responded by whealing. In other words, the whealing response to light does not prevent whealing in the same area. Similarly, a light wheal may be superimposed on a wheal formed by electrophoresis of 1:10,000 histamine.

The preceding observations, which will be reported subsequently in detail, do not support the point of view that histamine or a readily diffusible H-substance of low molecular weight is responsible for the skin response to ultraviolet irradiation.

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DISCUSSION

DR. SAMUEL J. PRIGAL, New York.—May I ask Dr. Abramson if he can prevent the whealing by administration of epinephrine or whether the whealing can be made to recede by injection of epinephrine?

DR. FRANCIS M. RACKEMANN, Boston.—I would like to ask Dr. Abramson if the capacity for whealing can be exhausted by repeated exposure.

DR. ARMAND COHEN, Louisville.—I wonder if Dr. Abramson will tell a little more about porphyrins and also the sulfanilamide type reaction. The porphyrins seem to be so much more potent than others.

DR. ABRAMSON.—In answer to the first question, I have prevented slight whealing to light by epinephrine locally, by administering epinephrine by electrophoresis. That is fairly easy to do. A piece of cotton is saturated with 1 per cent epinephrine phosphate at pH 5.0, and the positive pole is applied. Blanching occurs almost at once. Local vasoconstriction produced in this way leads to a retardation of whealing subsequent to irradiation. I have not made any quantitative study of this phenomenon, however. It is probable that some of the pioneer investigators in light hypersensitiveness have injected epinephrine. I believe that, if sufficient epinephrine were given, within limits, the reaction could be markedly retarded, but I doubt if it could be prevented completely in this patient.

Whether a light wheal can be exhausted by repeated exposures is an interesting question, but I am afraid I cannot answer it. The wheals which are produced by light last so long that it would be difficult to give repeated exposures before pigmentation itself would lead to some retardation. One of the difficulties, of course, which I have not mentioned is that a standard source of ultraviolet light has not been available. That has prevented quantitative work.

As far as the wheals produced by cold and heat are concerned, I have tried to get histamine from a cold wheal by reversed electrophoresis, but I regret to say that my results are merely preliminary. Dr. Maisel and I have worked on that subject, and we have found that neither histamine nor histaminase prevent whealing in cold hypersensitiveness when studied *quantitatively* by means of the contour gauge in one case. The pseudopod in a cold wheal may not be a true pseudopod. If a circular piece of metal is fitted to the bottom of an open tube and tube itself is filled with cracked ice, it is possible to apply a cold stimulus through an area well demarcated. Pseudopods of the usual type have not been obtained with a standard cold stimulus applied in this way. The gradients of heat leading up to the edge of the test tube are uniform, and we get a fairly circular wheal which, although not as sharply demarcated as the light wheal, does not have the typical pseudopod of histamine or ragweed wheals. Irregularities may be due to temperature gradients in the skin formed during the application of cold.

The discussion of the porphyrins is very involved. Briefly, there is no evidence that the porphyrins are responsible for urticaria solare or for most cases of light hypersensitiveness although there is evidently some relationship at times between the two.

Sulfanilamide is a different kind of light hypersensitiveness. It is photodynamic sensitization, and, as Blum has shown, it requires oxygen. It bears no obvious relationship to urticaria solare.

Studies of the Expectorant Action of Iodides. Louis Tuft, M.D., and Nathaniel M. Levin, Philadelphia, Pa. (Abstract.)

Clinical use of expectorants is widespread but on an empirical basis. What little is known of their actual mode of action has been obtained almost solely from experiments in animals, as indicated by review of literature. The present study originated from a desire to find the most effective expectorant drug capable of liquefying the viscid bronchial secretion of asthmatic patients. It was thought that such information could be obtained by the use of the bronchoscope. The general plan of procedure was to administer a drug intravenously and orally, obtain the bronchial secretion by aspiration, and then study it chemically. Iodides were chosen first because their presence could be more easily determined chemically. Accordingly, six patients were given from 15 to 31 grains of sodium iodide intravenously, and bronchoscopic specimens were obtained before the injection and at five-minute intervals thereafter for as long as it was possible to retain the bronchoscope. The specimens were then examined chemically for the presence of iodides. In some of the patients salivary secretions were obtained coincidentally and similarly examined for iodides; also in some, urine specimens were collected before and at intervals after the injection. A second group of nine patients was given 1 drachm of a saturated solution of potassium iodide. Specimens of bronchial secretion were then obtained at varying intervals thereafter, depending upon the time the bronchoscope was inserted, so as to get some comparative idea as to the appearance time after oral administration.

It was found from the results of these experiments that, although the time was somewhat variable, the iodides generally appeared in the bronchial secretion within fifteen to twenty-five minutes after intravenous injection and possibly slightly slower after oral administration. Salivary excretion occurred rather promptly, but urinary excretion was distinctly delayed. This may be taken to indicate that, as in animal experiments, the salivary glands and bronchi act as selective excretory organs for iodides and that urinary excretion must await reabsorption from the bronchi and gastrointestinal tract. These experiments demonstrate, therefore, for the first time in the human being that iodides given either orally or intravenously are excreted into bronchial secretion within fifteen to twenty-five minutes. We believe that this excretion

probably accounts for the secretion of iodides as expectorants and that oral administration probably is just as effective as intravenous and less troublesome.

DISCUSSION

Dr. Walter S. Burrage, Boston.—Up to the present time less has been known about the physiologic action of iodides than of any other drug used in allergy. I have recently had occasion to review the contents of all of the common nostrums and have found that over 90 per cent of them contain iodides. Potassium iodide is undoubtedly one of the best drugs we have in allergy. In certain types of cases plenty of fluids should be given with it.

May not the beneficial action of iodides be due in part to the ease with which they conjugate with and thereby alter the specificity of offending allergens?

Dr. Charles N. Hensel, St. Paul.—I should like to ask the essayist a question. In the study on the different effects of iodine administered intravenously and orally, was the iodine given on a full or empty stomach when it was given by mouth?

Dr. Alexander Sterling, Philadelphia.—I should like to ask Dr. Tuft whether he noticed, while doing this work, the effect of iodides upon the nasal secretion. For years I have noticed that the first effect of an overdose of iodides is an extreme rhinitis.

Dr. Tuft.—I cannot answer Dr. Burrage's question as to the conjugation of iodides. We were interested only in whether the iodides were excreted at all and not in the mechanism.

As to Dr. Hensel's question, we did administer the iodides on an empty stomach. All these patients were bronchoscoped, and this naturally was done without any food being given before the bronchoscopy.

I cannot answer Dr. Sterling's question because in our experiments we were interested only in the bronchial and the oral secretions and not in the nasal secretions.

Selected Abstracts

Asthma and Hay Fever

UNDER THE DIRECTION OF SAMUEL M. FEINBERG, M.D., AND THEODORE B. BERNSTEIN, M.D., CHICAGO

Vitamin C (Ascorbic Acid) Nutrition in Bronchial Asthma. An Estimation of the Daily Requirement of Ascorbic Acid. Goldsmith, G. A., Ogaard, A. T., and Gowe, D. P.: Arch. Int. Med. **67**: 597, 1941.

The authors found that the amount of ascorbic acid in the blood plasma in a group of twenty-nine persons with bronchial asthma varied from 0.02 to 1.87 mg. per 100 c.c. and averaged 0.410 ± 0.051 mg. per 100 c.c. In nineteen instances a subnormal plasma level was found. In a comparable control group of forty-three patients, the ascorbic acid values ranged from 0.07 to 2.40 mg. per 100 c.c., with a mean value of 0.602 ± 0.049 mg. per 100 c.c.

Seven asthmatic persons were placed upon a standardized ascorbic acid regime, which consisted of the intake of large amounts of ascorbic acid, for several months. Six of these patients were unable during this time to maintain a level of 1.0 mg. or more of ascorbic acid per 100 c.c., whereas members of the previously studied control group maintained such a level under the same regime in most cases. The authors interpret this finding to indicate an increased requirement of vitamin C in patients with asthma.

That there was a relationship between the level of ascorbic acid in the blood and the severity and frequency of the asthmatic attacks was indicated in two patients. When the blood was saturated with vitamin C, the condition of these two patients was much better. In five other patients the asthma was not influenced appreciably by the administration, and any beneficial effect noted was probably due to other causes.

The Histamine Treatment of Allergic Diseases. I. Asthma and Vasomotor Rhinitis. Farmer, L.: J. Lab. & Clin. Med. **26**: 802, 1941.

Observations to the effect that the symptoms of experimental anaphylaxis and clinical allergy are caused by the release of histamine or a histamine-like substance from cells, led the author to treat a series of allergic persons suffering from asthma, vasomotor rhinitis, or both with histamine. He based his therapy upon the theory that it is possible to produce refractoriness to histamine by repeated parenteral injections of histamine. The majority of the patients gave positive skin reactions to one or more allergens. When elimination of the positively reacting allergens did not cause relief, the patients were given histamine. Relatively small doses were used. The first dose generally in mild cases was 0.1 gamma (0.0001 mg.) of histamine phosphate, while in the severe cases 0.01 gamma was given. In the beginning, the injections were given two or three times weekly and were increased each time by 50 per cent if well tolerated. Later, treatment was given at five-, seven-, ten-, fourteen-, and twenty-one-day intervals. The maximum dose was 50 to 75 gamma. With marked reactions, or when the injection was not well tolerated, a smaller amount was injected the next time. The same precautions were observed as in

specific desensitization. The author reported very encouraging results. Of twenty-three patients, only five showed no improvement.

A Pollen Survey of the Islands of Bermuda. Gay, L. N., Curtis, H., and Norris, T.: Bull. Johns Hopkins Hosp. **68:** 179, 1941.

This survey covers the period from March 1, 1939, to April 1, 1940. The only three pollens found on the slides were those of the native cedar (*Juniperus bermudiana*) and the candleberry-myrtle (*Myrica cereferal*). Pollen of the cedar was found on all slides exposed during all but three months of the year, September, October, and November, but the peak weeks of pollination were during the month of March and the first week in April; the number of grains of pollen during this time averaged from 1,000 to 7,000 grains per square centimeter. It is only during these weeks that the cedar pollen-sensitive patient had clinical symptoms. The candleberry-myrtle tree pollinates during the month of March, and so far the authors have not encountered a case of clinical sensitivity. Grass pollinates from May to September, but grass pollen hay fever is of no significance on the island, because of the small amount of pollen in the air. The highest count was on June 20, with a total of eleven grass pollen grains per cubic centimeter. The same situation exists for plantain pollen, with a peak count of 5 grains per cubic centimeter on October 28. No ragweed pollen was found on the slides at any time; neither have any specimens of the plant been encountered on the islands. The authors suggest Bermuda as a haven of refuge for those sensitive to ragweed, grass, and plantain pollens. These islands are only forty-two hours by boat, and four hours by plane, from New York.

The Action of Parasympathetic-Mimetic Drugs in Asthma. Moll, H. H.: Quart. J. Med. **9:** 229, 1940.

The author made observations on the effect of parasympathetic-mimetic drugs in a series of cases of asthma and other chronic respiratory diseases. The drug used was acetyl-beta-methylcholine (mecholyl). Asthmatic subjects, of which there were twenty-eight, almost invariably reacted to the injection of 10 to 20 mg. of the drug with respiratory symptoms which varied in intensity from slight wheeziness to a severe asthmalike attack. Control subjects did not develop chest symptoms but showed the general signs of parasympathetic stimulation (flushing, sweating, salivation, and lacrimation). The peculiar tropism of the choline derivatives for the bronchial system seems to be due to the increased sensitivity of the bronchial nerve endings or of the bronchial muscle itself. Lung damage is an essential factor in determining the abnormal bronchial response. The test with mecholyl does not help in the differentiation between the various types of asthma; it may, however, assist in the diagnosis between true bronchial asthma and respiratory neurosis (hysterical hyperpnea). The asthmalike attack produced by choline derivatives is similar to, but probably not identical with, spontaneous asthma.

Changes in Serological Reactions and Tissue Sensitivity in Hay Fever Patients During the Early Months of Treatment. Sherman, W. B.: J. Immunol. **40:** 289, 1941.

The sera of thirteen ragweed hay fever patients were studied by the author with respect to the changes produced after three months of treatment with pollen extracts. Corroborating the findings reported by others, the results showed that in twelve of the thirteen patients the reactions to intracutaneous testing with ragweed extract was less after treatment; in eleven patients, reactions to conjunctival tests

were also less after treatment. As determined by the serum dilution method, an increase in skin-sensitizing antibody was found in twelve sera after treatment, and in all thirteen sera when the neutralization method was used. The presence of the blocking or reaction-inhibiting antibody was observed in all thirteen post-treatment sera by the use of the method that has been described by Loveless. The specificity of this antibody was also confirmed.

Another way of testing for the blocking antibody is described. It is a serum dilution method, in which a fixed amount of ragweed extract is added to heated before- and after-treatment serum, and serial dilutions are made. The mixtures are used to test sites previously made with unheated serum from an untreated case of ragweed hay fever. However, the results obtained with this method were not always in accordance with those obtained with the neutralization method. These discrepancies were explained when it was found that, although all the sera gave positive passive transfer reactions to Fraction 1 of a ragweed extract (albumin-precipitated by half saturation with ammonium sulfate from a water extract of low ragweed pollen), only six reacted also to Fraction 2 (precipitated by complete saturation after the removal of Fraction 1), and, following treatment, blocking antibody for Fraction 2 was formed in one serum only. Consequently, in testing for blocking antibody by this serial dilution method, the results obtained depended upon the way in which the serum used to sensitize cutaneous sites reacted to the two antigenic fractions of the ragweed pollen.

Clinical results were equally satisfactory in patients sensitive to Fraction 1 as compared to patients sensitive to both fractions, but those who were sensitive to Fraction 2 and did not form blocking antibody for that fraction did not develop tolerance to large doses of ragweed pollen extract and had more constitutional reactions than did those who were sensitive to only one fraction and developed an antibody inactivating that fraction.

Dermatology

UNDER THE DIRECTION OF MARION SULZBERGER, M.D., NEW YORK, AND JOSEPH GOODMAN, M.D., BOSTON

Barbituric Acid Dermatitis With Photosensitization. Stryker, G. V.: J. Missouri M. A. **36**: 484, 1939.

The 2-year-old patient discussed in this report had been given barbiturates for six weeks for epilepsy. Ten days before she was seen an eruption developed on the face and soon spread to the rest of the body. After the administration of the drug was stopped, the eruption subsided rapidly.

Three months later the eruption suddenly recurred, within twenty-four hours after exposure to the sun in the early spring. The dermatitis was sharply limited to the exposed areas. Small quantities of barbital and porphyrin, which were found in the urine at the time of the recurrence, gradually decreased in quantity. There was no recurrence in the next four months.

Skin, Conjunctival, and Scleral Reactions in the Course of Therapy With Sulfathiazole. Haviland, J. W., and Long, P. H.: Bull. Johns Hopkins Hosp. **66**: 313, 1940.

The authors describe a new type of reaction to sulfathiazole, a reaction which has not been reported after administration of other members of the sulfonamide group. Of seventy-eight patients treated with sulfathiazole, six presented conjunctival and scleral injection, restricted mainly to the exposed portion of the bulbar

conjunctiva and sclera. In four of the six cases there was an associated cutaneous eruption. It appeared that the severity of the reaction was proportional to the amount of light exposure.

Industrial Dermatitis. Klauder, J. V.: Indust. Med. **9**: 221, 1940.

The author discusses various approaches to the problem of the control of industrial dermatitis. Among the most important are education of the employee in proper methods of protection and cleansing and the furnishing of proper cleansing agents and accessories. Mechanical protection (gloves, etc.) is to be preferred to protective hand creams. Soaps containing abrasives, excessive cocoanut oil, and free alkali are to be avoided. Proper methods of cleansing are given for various exposures.

Exfoliative Dermatitis Due to Phenobarbital With Fatal Outcome: Report of Two Cases. Wile, U. J., and Benson, J. A.: Ann. Int. Med. **13**: 1243, 1940.

Two fatal cases of exfoliative dermatitis caused by phenobarbital are reported. The literature is reviewed.

Contact Dermatitis From Vegetation. Shelmire, B.: South. M. J. **33**: 337, 1940.

The author describes his method of preparing plant extracts for patch testing. Dilutions of the extract are made up in acetone. The tests, which are not covered, are applied by moistening the cork of the extract bottle and touching it to the skin. Sixty tests may thus be applied in five minutes.

Five to thirty drops of a 1:25 dilution of poison ivy extract in corn oil were given by mouth (in capsules) to nineteen persons who had had attacks of poison ivy dermatitis and who gave positive patch test reactions to the extract. In all of this group, reactions to the oral extract developed. These reactions consisted of flares of the skin and mucous membrane patch test sites, dermographism, pruritus ani, vesicular eruptions on the hands, mild diarrhea, and erythematous and urticarial eruptions.

One week after the disappearance of the skin manifestations, fourteen of this group were again given the same dose or double the initial dose. After the second dose only one patient presented a reaction, a mild pruritus ani. Patch tests in serial dilutions showed a marked decrease in sensitivity to the poison ivy extract.

Immunology

UNDER THE DIRECTION OF MATTHEW WALZER, M.D., AND MAX HARTEN, M.D., BROOKLYN

Studies on the Mechanism of the Arthus Phenomenon. Cannon, P. R., and Marshall, C. E.: J. Immunol. **40**: 127, 1941.

The authors undertook the investigation of the Arthus phenomenon using crystalline egg albumen as the antigen and the method of agglutination of collodion particles for the determination of the precipitative potency of the serum.

Specific precipitins were demonstrated in the serum at the time of the cutaneous test in every instance in which the Arthus phenomenon was produced. A marked parallelism between the precipitative strength of the rabbit serum and the intensity of cutaneous reactivity in the Arthus phenomenon could be demonstrated.

The development of the Arthus reaction required not only the presence of antibodies but also the ability of the animal to respond actively by inflammation to cellular or tissue injury. Young animals may have relatively high precipitin titers

but a low degree of cutaneous reactivity to inflammatory stimuli. The injection of large quantities of specific antigen into sensitized rabbits (partial desensitization) leads to a corresponding drop in the precipitative strength of the serum and a marked decrease or disappearance of cutaneous reactivity. With subsequent rise in precipitative titer cutaneous hypersensitivity reappears. Cutaneous hypersensitivity of rabbits passively sensitized with highly sensitive serum varied directly with the amount of serum injected. The removal of precipitins from such sera by specific antigenic absorption resulted in a loss of power to confer passive sensitization.

The Arthus reaction could not be elicited satisfactorily in the white rat or the cat, animals which do not readily form precipitins.

The data presented suggest that the Arthus phenomenon is dependent upon the union of circulating precipitin and its specific antigen within tissues.

Studies on Photo-Oxidation of Antigen and Antibodies. Smetana, H., and Shemin, D.: J. Exper. Med. **73**: 223, 1941.

Quantitative precipitin studies with recrystallized egg albumen, before and after photo-oxidation, indicated a progressive loss of antigenic function following such treatment. Prolonged photo-oxidation of antisera, e.g., rabbit anti-egg albumen serum and antipneumococcus Type I horse serum, resulted in a progressive lowering of the precipitative potency of these sera. The globulin gamma fraction of anti-egg albumen rabbit serum was separated by electrophoresis and exposed to light in the presence of hematoporphyrin hydrochloride. A Felton solution of antipneumococcus Type I horse serum was similarly photo-oxidized. Quantitative precipitin reactions revealed that these specific antibody fractions were affected by photo-oxidation to the same degree as were the antibodies in whole sera.

Egg albumen, when photo-oxidized to a degree where its precipitin reaction is destroyed, loses its antigenicity and no longer produces anaphylactic shock in sensitized animals. Similarly, photo-oxidized egg albumen does not induce precipitin formation in rabbits. Photo-oxidation of egg albumen destroys the antigenicity of the protein and does not produce an altered protein.

The results of chemical studies on the effects of photo-oxidation on the various amino acid constituents of egg albumen are reported as are the altered electrophoretic patterns resulting from photo-oxidation of egg albumen, antisera, and specific antibody fractions.

Acute Edema of the Pancreas, Gall Bladder, and Stomach in Serum Anaphylaxis. Auer, J., and Krueger, H.: Proc. Soc. Exper. Biol. & Med. **46**: 74, 1941.

Acute Edema of the Pancreas, Gall Bladder, and Stomach After Histamine Injections. Auer, J., and Krueger, H.: Ibid. **46**: 75, 1941.

Dogs, sensitized by one or more parenteral injections of 5 ml. of horse serum, were reinjected intravenously twenty-one to thirty-eight days later with 20 ml. of horse serum. In another series, from 2 to 6 mg. per kilogram of histamine hydrochloride were infused into the jugular vein. In both studies biopsy specimens of pancreas, gall bladder, and stomach were obtained thirty minutes after the administration of the shocking dose.

In animals shocked twenty-one days after the sensitizing injections, the authors observed edema of the pancreas eight times, edema of the gall bladder mucosa and adventitia fifteen times, and edema of the stomach submucosa eight times. The degree of edema varied in different animals. In some instances the entire pancreas was involved so that the lobules were separated by clear, watery fluid. In the gall bladder, the edema of the mucosa and adventitia involved the fundus chiefly. In

the stomach, the edema was noted mainly in the submucosa of the antrum and was most marked along the lesser curvature. The duodenal submucosa showed no appreciable edema.

Essentially the same findings were observed in histamine shock in dogs.

Studies Concerning Possible Mechanisms Involved in Experimental Serum Sickness. Jones, L. R., and Roberts, E. C.: J. Immunol. **40**: 107, 1941.

The parenteral administration of histaminase did not affect the incidence or duration of experimental serum sickness in the rabbit.

The administration of a single dose of rabbit antihorse serum, obtained from rabbits at various times after the termination of serum sickness, did not influence the incidence or course of experimental serum sickness.

The subcutaneous injection of rabbit antihorse serum into rabbits prepared by the injection of 0.1 ml. of horse serum into the ear elicited a reaction at the ear site within three to six hours. The antigen-injected tissues were capable of reacting soon after the injection and remained so for four to six days thereafter. The reaction, termed local serum sickness by the writers, was characterized by erythema and edema which persisted for several hours and then subsided without residual tissue injury. This local serum reaction was not elicited by heat-alkali processed horse serum when comparable amounts of antiserum were administered. Reactions were, however, elicited with larger amounts of antiserum. The authors conclude that the heat-alkali treatment leads to a quantitative reduction rather than to an elimination of the qualitative factor in horse serum upon which experimental serum sickness in the rabbit seems to depend.

The Treatment and Prevention of Serum Sickness by the Use of Histaminase. Cherry, J. H., and Prickman, L. E.: Proc. Staff Meet., Mayo Clin. **16**: 38, 1941.

Ten of twelve patients showed definite improvement following the daily oral administration of 5 or 6 tablets or capsules of histaminase after serum sickness had started. In four of eight patients to whom histaminase was given orally in an effort to prevent the occurrence of serum sickness no reaction developed, while in the others only mild transitory serum disease occurred.

The authors realize the limitations of the investigation. Final evaluation of this remedy awaits a study of its effectiveness in a controlled large group of patients.

Rhinology and Ophthalmology

UNDER THE DIRECTION OF FRENCH K. HANSEL, M.D., ST. LOUIS

Sinusitis in Children. Beck, J. C., and Fabricant, N. D.: Am. J. M. Sc. **199**: 865, 1940.

In discussing the matter of sinusitis in children, Beck and Fabricant emphasize the importance of recognizing allergy as the possible cause of sinus disease, and its differentiation from pure sinus infections.

Species Specificity of Proteins of Optic Lens. Markin, L., and Kyes, P.: J. Infect. Dis. **65**: 156, 1939.

In experimental studies of the species specificity of proteins of the optic lens, Markin and Kyes found that the extreme specialization of the optic lens results in a tissue whose constituent proteins are the same in two mammalian species as

widely separated as dog and beef and are therefore in full accord with the results obtained with the precipitation tests by Uhlenhuth, Hektoen, and others. Markin and Kyes further observed that the autosensitization to lens proteins induced in the guinea pig is not noted in the pigeon.

Analysis of Thirty Consecutive Cases of Allergic Conjunctivitis. Thomas, J. W., and Warren, W. A.: Cleveland Clin. Quart. **7**: 3, 1940.

Thomas and Warren present an analysis of thirty consecutive cases of allergic conjunctivitis. Results of skin tests concurred with the history of inhalant factors responsible for the eye symptoms in fourteen cases. Only six of the thirty patients, or 20 per cent, had no related allergic manifestations. Satisfactory results were obtained in more than 50 per cent under allergic management. This group of cases was not selected but consisted of thirty consecutive cases of allergic conjunctivitis with no exceptions.

Respiratory Allergy: Survey of 283 Consecutive Cases Seen in Office Practice From July, 1937, to December, 1938. Halpin, L. J.: J. Iowa M. Soc. **29**: 439, 1939.

Halpin reviews his studies of 283 consecutive cases of respiratory allergy seen in private office practice from July, 1937, to December, 1938. They were classified as follows: seasonal hay fever, 71; seasonal bronchial asthma, 66; perennial nasal allergy, 94; and perennial bronchial asthma, 52. Adequate allergic investigation was completed in 274 cases. Encouraging results were secured in the majority of the patients. The importance of a thorough allergic investigation to determine the etiological factors is emphasized.

Atopic Cataracts. Beetham, W. P.: Arch. Ophth. **24**: 21, 1940.

Beetham states that allergic cataract may be unilateral as well as bilateral. In 30 to 50 per cent of the cases involvement is unilateral. Ten cases are described in detail. On two occasions intradermal tests with aspirated aqueous humor gave negative reactions. In two cases histologic examination of the crystalline lens showed cortical degeneration and normal capsule. It is noteworthy that all the patients had an active, long-standing eczema; two also had asthma. Seven of the patients were in the third decade. One patient was 40 years of age, and two were 4 and 16 years, respectively. Five were males, and five were females. The possible part played by infection, avitaminosis, deficiency of the endocrine glands, and disturbance of the autonomic nervous system were discussed. In the opinion of Beetham, allergy appears to be the most important etiological factor.